to give 4 (0.141 g, 72%): mp 137 °C; UV (95% EtOH) λ_{max} 298 nm (log ϵ 3.93), 262 (sh, 4.25), 252 (4.40); λ_{min} 275 nm (log ϵ 3.70) 230 (3.90); ^{1}H NMR ($CDCl_3$) δ 7.36 and 7.30 (2 H, s, 5-H and 6-H), 6.19 (1 H, br s, 3-H), 4.75 (2 H, s, 4'-CH_2O), 3.48 (3 H, s, OCH_3), 2.56 and 2.48 (6 H, s, 4-CH_3 and 5'-CH_3). Anal. ($C_{15}H_{14}O_4$) C, H.

Complexes in the Ground State. Binding experiments were performed in aqueous solutions containing 1.5×10^{-2} M NaCl and 1×10^{-3} M EDTA. Dialysis experiments,[13] as well as fluorimetric titrations,[14] are described elsewhere.

Computation of the Interaction Parameters. The method of computation involved an iterative procedure designated to satisfy the following equation of Mc Ghee and Von Hippel:[16]

$$\frac{r}{c} = K(1 - nr)\left(\frac{1 - nr}{1 - (n - 1)r}\right)^{n-1}$$

given the experimentally determined values of r and c and the initial guess of K (the intrinsic binding constant to an isolated site) and of n (the number of nucleotides occluded by a bound furocoumarin molecule). The program, based on the least-squares method of the Taylor series expansion of the above-reported equation, was made to recycle until K and n changed by less than 1% and then to give the final values of K and n with a calculated binding isotherm at 5% saturation increments.

Irradiation Procedure. To aqueous solution (containing a 2×10^{-3} M NaCl) of DNA (2.3×10^{-3} M) was added of 4.7×10^{-5} M compound to be examined; the concentration of the angelicins was always checked by radiochemical measurements and, if necessary, was corrected. Irradiation was made in a test tube immersed in a thermostatically controlled cell; the irradiation intensity, determined by means of a chemical actinometer,[28] was 1.07×10^{16} quanta s^{-1} mL^{-1}. After irradiation, solid NaCl was added until 2 M and 2 volumes of absolute ethanol were added; precipitated DNA, collected by centrifugation, was washed with 80% ethanol and redissolved in the initial volume of water.

Radioactivity Measurements. A liquid scintillation spectrometer (Packard Model 3375) was employed. Dioxane base (PPO, 5 g; POPOP, 0.075 g; naphthalene, 120 g; dioxane, up to 1000 mL of solution) or toluene base (PPO, 5 g; POPOP, 0.05 g; toluene, up to 1000 mL of solution) scintillators were used.

Evaluation of Cross-linkages. This evaluation was made directly on the irradiated DNA samples, without precipitation, accordingly to reference procedures.[22]

Fluorimetric Measurements. Fluorimetric measurements were carried out on part B of the irradiated samples after acidic hydrolysis,[21] by means of a spectrophotofluorimeter (Perkin-Elmer, Model MPF-044).

Flow Dichroism Measurements. Flow dichroism measurements were carried out by means of a Schimadzu QV-50 spectrophotometer with a flow dichroism attachment, which replaces the usual cell chamber and consists of a quartz cylindrical cell containing a quartz rotating cylinder and a calcite prism that polarizes the monochromatic light parallel (||) and perpendicular ($\perp$) to the flow line.[29] Linear flow dichroism as well as the reduced dichroism were calculated according to Wada[30] and Wada and Kozawa.[29]

DNA and RNA Synthesis. Ehrlich ascites tumor cells in Hank's solution (aliquots of 5 mL; 2×10^7 cells/mL) were irradiated in petri dishes (5 cm in diameter) placed on ice by a Philips HPW 125 set at 20-cm distance (2.02×10^{16} quanta/s incident on the whole sample). Samples of 2×10^6 of the irradiated cells in 0.5 mL in the same medium containing 1 μCi of [^{3}H]thymidine or of [^{3}H]uridine were incubated for 15 min at 37 °C. The reaction was stopped by chilling on ice and adding 1 mL of 5 mM unlabeled nucleoside. The cells were collected by filtering on Whatman CF/c dishes (diameter 2.5 cm), washed three times with saline, and treated with 10mL of ice-cold 10% trichloroacetic acid. After 1 min, the samples were filtered and washed six times with 10 mL of 10% trichloroacetic acid; the filters were dried and counted. Each UV-A radiation dose was studied at least in duplicate, while the controls were four samples of untreated cells. Results were calculated on the basis of the specific radioactivity incorporated into nucleic acids and expressed as percentage of the incorporation observed in the untreated cells (2000 dpm/μg of DNA and 2400 dpm/μg of RNA). DNA and RNA contents were determined by the diphenylamine[31] and orcinol[32] reactions. The data were submitted to probit analysis and expressed as the D_{50}, i.e., the UV-A radiation dose that in the presence of the 1.9×10^{-5} M produces a 50% inhibition.

T_2 Phage Inactivation. T_2 phage was grown using *Escherichia coli* B_{48} as host bacteria and the brain-heart infusion broth (from Difco Laboratories, Mich.); virions were irradiated in Hank's solution at a density of 10^{10} particles per milliliter, as reported above for the Ehrlich cells, and in the presence of 4 μg/mL of the tested compounds. Virus titers were determined according to Adams,[33] using the same host and the same medium.

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Potential Inhibitors of Nucleotide Biosynthesis. 1. Nitrosoureidonucleosides. 2

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Several nitrosoureidonucleosides (**9a**, **9b**, **11a**, **11b**, 18 and **20**) designed as inhibitors of enzymes that metabolize purine and pyrimidine nucleotides have been prepared and their chemical and biological properties studied. The low level of biological activity observed may be due to the unexpected stability of these nitrosoureas compared to biologically active compounds such as *N*-methyl-*N*-nitrosourea (MNU), *N*,*N*'-bis(2-chloroethyl)-*N*'-nitrosourea (BCNU), and *N*,*N*'-dicyclohexyl-*N*-nitrosourea (DCyNU).

Except for the early reactions of the biosynthetic pathway to uridylic acid, all of the enzymatic reactions leading to ribonucleotides of RNA and the 2'-deoxyribonucleotides of DNA take place at the mono- and diphosphate levels.[1,2] The phosphate moieties of the compounds involved are important to their binding to the active site of the enzymes that carry out these transformations. Almost all of the known inhibitors of these enzymes are nucleoside mono- and diphosphates. These compounds, however, are labile

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under in vivo conditions in the presence of the various phosphate-cleaving enzymes. Despite data obtained under in vitro conditions after enzyme inactivation,[3] the overwhelming body of in vivo data strongly indicates that the therapeutic potential of intact nucleotides is nil. They cannot enter cells except perhaps extremely slowly, and they are readily cleaved extracellularly to the nucleoside.[4-6]

Because of this transport problem, all of the purine and pyrimidine antimetabolites that are active in vivo are administered as the free bases or as nucleosides, both of which must be metabolically activated by conversion intracellularly to the nucleotides. Examples are the 6-thiopurines, 5-fluorouracil, 5-azacytidine, and the arabinonucleosides *ara*-C and *ara*-A. Resistance to these agents frequently results from the selection of a mutant cell population deficient in the activating enzymes, i.e., the phosphoribosyltransferases or the kinases.[7] Attempts, so far largely unsuccessful, to circumvent this problem have until now consisted of efforts to find and attach to nucleosides groups that are able to simulate the binding properties of the phosphate group and yet allow compounds containing them to enter cells intact[8] or to prepare nucleotide derivatives that can enter cells and be cleaved intracellularly to the active nucleotides.[9-13]

There is another possible approach that attempts to turn the liability of the nucleotide or nucleotide analogue approach into an asset. X-ray crystallographic studies of the nuclease–Ca^{+2}–thymidine 3′,5′-diphosphate complex have established precisely the mode of binding of this nucleoside diphosphate to the enzyme active site.[14,15] These studies clearly show that the phosphate oxygens are hydrogen bonded to the guanidinium residues of arginine. In another instance, the binding of D-2,3-diphosphoglycerate to deoxyhemoglobin has been shown to occur through bonds to the imidazole ring of histidine residues, the ω-amino groups of lysine residues, and the α-amino group of valine.[16] Furthermore, reaction of cyanate with the amino groups blocks the interaction between DPG and amino groups by forming ureido groups in their place (hence, the inhibitory effect which cyanate has on the sickling process may be due to a reduction of the affinity of the deoxyhemoglobin S for DPG). Thymidine 3′,5′-diphosphate properly substituted with either the diazophenyl[17] or bromoacetamidophenyl[18] function becomes an excellent affinity label of the active site of nuclease. The imidazole ring of histidine, the phenolic hydroxy of tyrosine, and the amino group of lysine were the points of attachment of the alkylating or coupling moiety. Thus, the idea of a chemically reactive function properly positioned to react with the binding sites for the phosphate moieties of nucleoside mono-, di-, and triphosphates is already established as being fundamentally sound. These affinity-labeling experiments, however, were carried out at pH 8.8 and 9.4, unattainable in vivo. Further, pH studies showed that the rate of reaction increased rapidly with an increase in pH from 7 up to 10 and that at pH 7.4 the reaction and concomitant inactivation of the enzyme occurred slowly. This phenomenon results from the fact that since the phenolic group of tyrosine and the basic groups of lysine and arginine are fully protonated at pH 7.4,[19] they are poor nucleophiles for the attack of cations (or S_N2 reactions) at this pH. At the same time, it should be possible to alkylate the imidazole ring and α-amino groups, which are reactive at pH 7.4, at a reasonable rate. Furthermore, as stated above, cyanate is known to react in vivo with protonated amino groups, as do the isocyanates generated in vivo from nitrosoureas.[20] In fact, cyclohexyl isocyanate generated from *N*-(2-chloroethyl)-*N*′-cyclohexyl-*N*-nitrosourea (CCNU) is an active-site-specific inactivator of chymotrypsin.[21]

By means of Dreiding stereomodels, the binding to a guanidinium group of the phosphate of a nucleoside mono-, di-, or triphosphate can be simulated, and the proper placement of a potentially reactive carbon relative to the 5′ carbon of the nucleoside can be determined. By means of such modeling and considering the modes of binding known to occur, we have determined that a reactive carbon one and three atoms removed from the 5′ carbon should be in the proper position to react with the monophosphate binding sites. For a nucleoside diphosphate it should ideally be one, three, or five carbons removed. Because of the vagaries involved in such a determination (e.g., coiling of the side chain), it seemed prudent to plan to space the reactive center from zero to six atoms from the 5′ carbon (for mono- and diphosphate analogues), giving priority to the above-mentioned spacers.

Our first efforts to prepare this kind of inhibitor was directed toward the preparation of nitrosoureidonucleosides, a class of compounds recently described, although reported examples all have the nitrosoureido function attached directly to the sugar moiety of the nucleoside.[22-24] To prepare compounds with the nitrosoureido function spaced an appropriate distance from the C-5′, we elected to prepare the ribofuranuronic acids (**4a**, **4b**, and **14**) from

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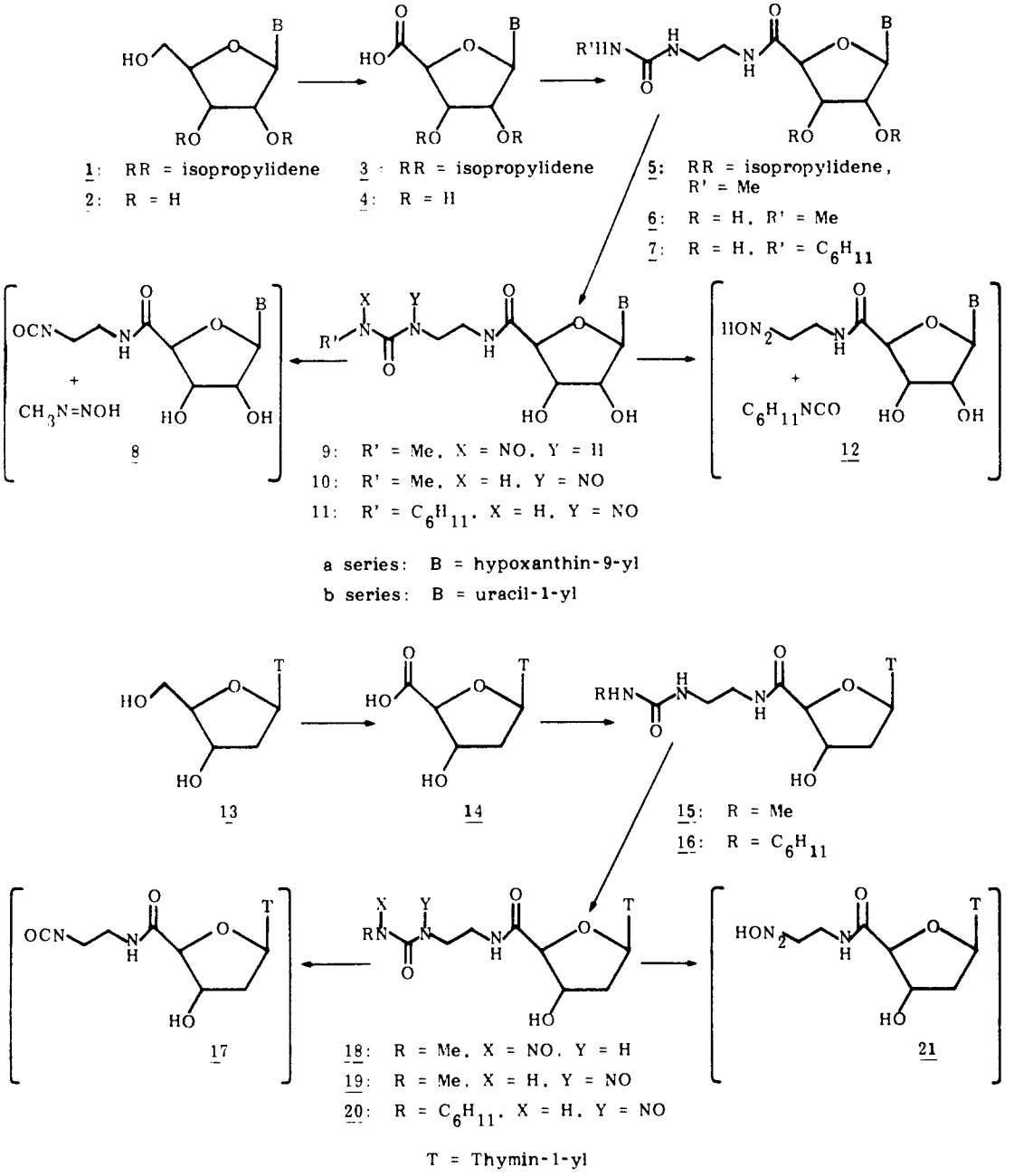
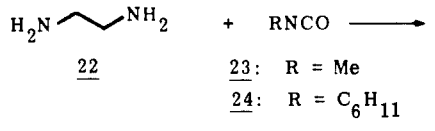
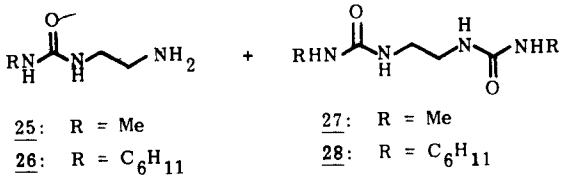
Scheme I



inosine,[25] uridine,[26] and thymidine[26] for conversion to amides of the (2-aminoethyl)ureas **25** and **26**. These ureas were prepared by the reaction of methyl and cyclohexyl isocyanates (**23** and **24**), with ethylenediamine (**22**), although efforts to eliminate the formation of the bisureas (**27** and **28**) were not successful. In fact, the relative amounts of mono- and bisureas reflected the relative reactivities of the two isocyanates.[27] Separation of the mono- and bisureas was accomplished by crystallization.





In the initial attempt to prepare the desired amide of the carboxylic acid derived from inosine, 2,3-*O*-isopropylideneinosine (**1a**) was oxidized to the acid **3a**, and isobutyl chloroformate was used to form the mixed anhydride for reaction with the amine (Scheme I). Lack of success with this approach led to the use of diphenylphosphoryl azide[28] with the isopropylidene derivative **3a**, which gave **5a**. Acid hydrolysis of **5a** gave the desired amide **6a**. Since the use of the azide does not require blocking the 2′,3′-hydroxyls, a later preparation of **6a** was

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Table I. Chemical Properties of the Nitrosoureidonucleosides

compd	$T_{1/2}$, h	alkylating act.: OD at 540 nm, 2 h at 37 °C	alkylating act.: OD at 540 nm, 0.5 h at 100 °C	carbamoylating act.
9a	127	0.031	0.217	1 205
9b	17	0	0.201	1 911
11a	12	0	0.354	9 210
11b	8	0	0.278	10 297
18	29	0.048	0.206	243
20	11	0	0.357	8 862
29[a]	45			
30[a]	11			
31[a]	40			
MNU	0.12	0.192	0.149	2 573
CCNU	0.88	0.198	0.857	17 283
DCyNU[b]	0.09	0.009		15 036

[a] 5'-Deoxy-5'-methylnitrosoureidoadenosine (**29**), 5'-deoxy-5'-methylnitrosoureidoinosine (**30**), and 5'-deoxy-5'-methylnitrosoureidouridine (**31**). See ref 23. [b] Data from ref 32, which also describes the methodology employed.

carried out on 1-deoxy-1-(1,6-dihydro-6-oxo-9*H*-purin-9-yl)-β-D-ribofuranuronic acid itself (**4a**), reducing the number of steps and raising the overall yield of **6a**. Variations of this procedure were then used to prepare **7a** and the amides of the acids from uridine (**6b** and **7b**) and thymidine (**15** and **16**); the yields ranged from 45 to 84%.

Nitrosation of **6a** was first carried out in aqueous acetic acid, which gave according to ^{1}H NMR (peak at 2.84 ppm, $NHCH_3$) about 15% of **10a**. The use of 95–97% formic acid reduced the amount of isomer to about 7%. The cyclohexylureas (**7a**, **7b**, and **16**) nitrosated in formic acid exclusively on the ethylene side to give **11a**, **11b**, and **20**, whereas the ^{1}H NMR spectra of the other methylnitrosoureas (**9b** and **18**) indicate that they contain about 6–8% of the isomeric nitrosourea (**10b** and **19**). In the case of the thymidine derivative **18** the identity of the isomer was confirmed by TLC (Greiss test), ^{1}H NMR, and MS and the amount by high-performance LC. Although the ^{1}H NMR cannot distinguish between nitrosation of N-1 of the urea group and N of the amide at C-5, the MS(EI) can, since fragmentation occurs between N-1 of the urea and CH_2 of the side chain and not between NH and CO of the amide. These results are in keeping with the known difficulty of nitrosation of amides.[29]

Chemistry. Previous studies[30] on the aqueous decomposition of N,N'-disubstituted *N*-nitrosoureas, including other nitrosoureidonucleosides,[23] have shown that breakdown occurs to give an isocyanate and a diazohydroxide (see **8**, **17**, **12**, and **21**). These unstable intermediates decompose further, in the absence of other nucleophiles, to give the corresponding amine and alcohol, respectively, although under certain conditions the isocyanate may react with the amine to give a symmetrical urea. Since the success of these potential enzyme inhibitors depends upon the generation of a reasonable concentration of these reactive intermediates (**8**, **17**, **12**, or **21**) under physiological conditions, studies on these compounds were carried out. The chemical half-lives, alkylating activities, and carbamoylating activities were determined (see Table I). The alkylating and carbamoylating activities were determined as previously described.[31,32] Since the UV method normally used for the determination of half-lives of nitrosoureas could not be used with these compounds because of the UV absorption of the purine and pyrimidine moieties, a satisfactory method was developed based on high-performance LC. Surprisingly, these nitrosoureas and some related ones previously prepared (**29–31**)[23] are much more stable and, thus, less reactive than the structural types previously shown to have biological activity,[31,32] with the methylnitrosoureido compounds being significantly more stable than the cyclohexyl compounds. The half-lives range from 8 to 127 h compared to the biologically active nitrosoureas such as *N*-methyl-*N*-nitrosourea (MNU), *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea (CCNU, log P = 2.8), 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose (chlorozotocin, log P = −1), and *N,N'*-dicyclohexyl-*N*-nitrosourea (DCyNU),[33] the half-lives of which range from 5.6 to 52.5 min. As a result, the alkylating and carbamoylating activities of these nitrosoureidonucleosides are lower than those of MNU, CCNU, and DCyNU.

Biological Evaluations. Compounds **6a**, **11b**, and **15** at 20 μg/mL inhibited the growth of H.Ep.-2 cells in culture slightly (to 75–80% of untreated controls); none of the others showed any effect. At 100 μg/mL, **9a** significantly inhibited the incorporation of hypoxanthine and guanine in RNA (61%) and DNA (35%), but none of the other inosine derivatives had much effect. Of the thymidine analogues, **15**, **16**, and **18** had some effect on the incorporation of uridine in RNA and DNA (20–30%). Compound **9a** did not significantly inhibit hypoxanthine phosphoribosyltransferase, even when incubated with the enzyme for 1 h before addition of substrate. Compounds **9a** and **20** were inactive against the P388 leukemia, whereas the uridine derivative **9b** was slightly active—26% ILS at 400 mg/kg and 28% at 200 mg/kg given daily for 9 days. The lack of biological activity of these compounds is not surprising in view of their lack of chemical reactivity.

Efforts are now being directed to the preparation of more reactive nitrosoureidonucleosides and nucleosides containing other functional groups, since attempts to thermally decompose **9a** to the diazohydroxide and isocyanate prior to biological evaluation were not successful.

Experimental Section

All evaporations were carried out in vacuo with a rotary evaporator. Analytical samples were normally dried in vacuo over P_2O_5 at room temperature for 16 h. Analtech precoated (250 μm) silica gel G(F) plates were used for TLC analyses; the spots were detected by irradiation with a Mineralight and by charring after spraying with saturated $(NH_4)_2SO_4$. Compounds containing amino groups were also detected with ninhydrin spray and those containing the nitrosoureido function with the Greiss reagent. All analytical samples were essentially TLC homogeneous. Melting points were determined with a Mel-Temp apparatus and are not corrected. The UV absorption spectra were determined in 0.1 N HCl (pH 1), pH 7 buffer, and 0.1 N NaOH (pH 13) with a Cary 17 spectrophotometer: the maxima are reported in nanometers ($\epsilon \times 10^{-3}$). The NMR spectra were determined with a Varian XL-100-15 spectrometer in Me_2SO-d_6 (unless otherwise specified) with tetramethylsilane as an internal reference: chemical shifts (δ) quoted in the case of multiplets are measured from the approximate center. The mass spectral data was obtained with a Varian MAT 311A mass spectrometer in the electron-impact (EI)

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or field-desorption (FD) mode. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Half-life Determinations. Solutions of the test compounds in pH 7 phosphate buffer or buffer plus DMF were prepared and held at ambient temperature with sampling at zero time and periodically thereafter. The aliquots were analyzed with a Hewlett-Packard Model 1084B liquid chromatograph using a 20 min gradient from 9:1 H_2O–MeCN to 1:9 H_2O–MeCN at a flow rate of 1 mL/min and with the variable wavelength detector set at 254 nm. Each compound gave one principal peak that indicated, after dissolutions, purities of 94–98% and that diminished with time with the appearance of two to three new major peaks. Since the decomposition of nitrosoureas is known to show apparent first-order kinetics, the half-lives were determined from semilog plots of the integrals of the principal peaks vs time. The urea precursors **15** and **16** were shown to be stable under the conditions used.

1-Deoxy-1-(1,6-dihydro-6-oxo-9*H*-purin-9-yl)-*N*-[2-(3-methylureido)ethyl]-β-D-ribofuranuronamide (6a). A suspension of 1-deoxy-1-(1,6-dihydro-6-oxo-9*H*-purin-9-yl)-β-D-ribofuranuronic acid[25] (**4a**; 1.41 g, 5.0 mmol), (2-aminoethyl)-methylurea (703 mg, 60 mmol), triethylamine (605 mg, 6.0 mmol), and diphenylphosphoryl azide[35] (1.65 g, 6.0 mmol) in DMF (50 mL) was stirred for 1 h in the cold (MeOH–ice bath) and then for 20 h at ambient temperature. The resulting clear solution was evaporated to dryness in vacuo to give a syrup that crystallized from H_2O: yield 1.48 g (78%); purity 91% (UV, TLC).

The analytical sample was previously obtained by the reaction of 2′,3′-*O*-isopropylidene derivative (**3a**) of the uronic acid (**4a**) as described above, followed by acidic hydrolysis of the isopropylidene group of **3a** to give the product **6a** as a crystalline solid: mp 162–165 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) 249 nm at pH 1 (11.92), 249 at pH 7 (12.15), 253 at pH 13 (13.08); TLC, H_2O; ^{1}H NMR δ 2.52 (m, CH_3), 3.15 (m, CH_2CH_2), 4.20 (m, $H_{3'}$), 4.33 (d, $H_{4'}$), 4.48 (t, $H_{2'}$), 5.4–5.8 (br, OH), 5.8 (d, CH_3NH), 5.97 (d, $J_{1',2'}$ = 7 Hz, $H_{1'}$), 8.15 (s, H_2), 8.43 (s, H_8), 8.53 (m, $CONHCH_2CH_2NHCO$); ^{13}C NMR δ 26.32 (CH_3), 39.0, 39.3 (CH_2CH_2), 72.85, 72.97 ($C_{2'}$,$C_{3'}$), 124.88 (C_5), 139.6 (C_8), 145.99 (C_2), 148.00 (C_4), 156.52 (C_6), 158.87 ($CH_3NHCONH$), 169.40 (CH_2CH_2NHCO). Anal. ($C_{14}H_{19}N_7O_6$) C, H, N.

1-Deoxy-1-(3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl)-*N*-[2-(3-methylureido)ethyl]-β-D-ribofuranuronamide (6b). Compound **6b** was prepared from 1-deoxy-1-(3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl)-β-D-ribofuranuronic acid[26] (**4b**; 876 mg, 3.36 mmol) in 45% yield (537 mg) by the procedure described above for **6a**. The analytical sample was obtained by recrystallization from water: mp 215–217 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) 260 nm at pH 1 and 7 (10.05), 261 at pH 13 (7.26); TLC $CHCl_3$–MeOH (3:1); ^{1}H NMR δ 2.68 (m, CH_3), 3.12 (m, CH_2CH_2), 4.10 (m, $H_{3'}$), 4.20 (m, $H_{2'}$), 4.29 (d, $H_{4'}$), 5.75 (d, H_5), 5.91 (d, $J_{1',2'}$ = 6 Hz, $H_{1'}$), 8.23 (s, H_6), 8.29 (NH); ^{13}C NMR 26.20 (CH_3), 72.66 and 72.75 ($C_{2'}$ and $C_{3'}$), 83.14 ($C_{4'}$), 88.04 ($C_{1'}$), 101.98 (C_5), 141.41 (C_6), 150.87 (C_2), 158.90 (NHCONH), 163.07 ($C_{4'}$), 169.88 (CONH); MS, *m/e* 358 [(M + 1)$^+$]. Anal. ($C_{13}H_{19}N_5O_7$) C, H, N.

***N*-[2-(3-Cyclohexylureido)ethyl]-1-deoxy-1-(1,6-dihydro-6-oxo-9*H*-purin-9-yl)-β-D-ribofuranuronamide (7a).** Compound **7a** was prepared from 1-deoxy-1-(1,6-dihydro-6-oxy-9*H*-purin-9-yl)-β-D-ribofuranuronic acid (**4a**; 564 mg, 2 mmol) as described above (preparation of **6a**). The analytical sample was obtained by recrystallization from EtOH: yield 657 mg (71%); mp indefinite; TLC $CHCl_3$–MeOH (3:1) (+5% acetic acid); UV λ_{max} ($\epsilon \times 10^{-3}$) 248 nm at pH 1 (10.84), 249 at pH 7 (10.94), 253 at pH 13 (11.80); ^{1}H NNR δ 1.15 and 1.6 (2 m, CH_2 of C_6H_{11}), 3.15 (m, CH of C_6H_{11} and CH_2CH_2), 3.4 (br, H_2O), 4.22 (m, $H_{3'}$), 4.35 (d, $H_{4'}$), 4.58 (m, $H_{2'}$), 5.85 (br, OH and NH), 5.99 (d, $J_{1',2'}$ = 6 Hz, $H_{1'}$), 8.18 (s, H_2), 8.50 (s, H_8), 8.55 (br, NH); MS, *m/e* 450 [(M + 1)$^+$]. Anal. ($C_{19}H_{27}N_7O_6 \cdot 0.7H_2O$) C, H, N.

***N*-[2-(3-Cyclohexylureido)ethyl]-1-deoxy-1-(3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl)-β-D-ribofuranuronamide (7b).** Compound **7b** was obtained from **4a** by the procedure described for **6a**. The product was recrystallized from MeOH: yield 757 mg (53%); mp 210–212 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) 259 nm at pH 1 (10.1), 260 at pH 7 (10.0), 260 at pH 13 (7.05); TLC $CHCl_3$–MeOH–HOAc (7:3:1); ^{1}H NMR δ 1.18 and 1.166 (2 m, CH_2 of C_6H_{11}), 3.11 (m, CH_2CH_2), 3.35 (CH of C_6H_{11}), 4.1 (m, $H_{3'}$), 4.18 (m, $H_{2'}$), 4.37 (d, $H_{4'}$), 5.68 (m, OH), 5.75 (d, H_5), 5.9 (d, $J_{1',2'}$ = 6 Hz, $H_{1'}$), 8.35 (d, H_6), 8.3 (br, $CONHCH_2$), 11.35 (br s, N_3H); MS, *m/e* 426 [(M + 1)$^+$]. Anal. ($C_{18}H_{27}N_5O_7$) H, N; C: calcd, 50.81; found, 50.38.

1-Deoxy-1-(1,6-dihydro-6-oxo-9*H*-purin-9-yl)-*N*-[2-(3-methyl-3-nitrosoureido)ethyl]-β-D-ribofuranuronamide (9a). Solid $NaNO_2$ (964 mg, 14 mmol) was added in portions to a cold (MeOH–ice bath) solution of 1-deoxy-1-(1,6-dihydro-6-oxo-9*H*-purin-9-yl)-*N*-[2-(3-methylureido)ethyl]-β-D-ribofuranuronamide (1.48 g, 3.88 mmol) in formic acid (7 mL). The resulting solution was stirred with cooling for 0.5 h before evaporation in vacuo to a yellow syrup that crystallized from H_2O: yield 703 mg (44%); mp indefinite; UV λ_{max} ($\epsilon \times 10^{-3}$) 248 nm at pH 1 (16.0), 245 at pH 7 (16.1), 253 at pH 13 (13.3); TLC, BuOH–H_2O (6:1) and $CHCl_3$–MeOH–HOAc (17:3:1); ^{1}H NMR δ 2.84 (d, $NHCH_3$ of **10a**), 3.05 (s, CH_3), 3.33 (s, H_2O), 3.45 (m, CH_2CH_2), 4.2 (m, $H_{3'}$), 4.35 (d, $H_{4'}$), 4.54 (m, $H_{2'}$), 5.54 (d, $O_{2'}H'$), 5.73 (d, $O_{3'}H$), 5.96 (d, $J_{1',2'}$ = 8 Hz, $H_{1'}$), 8.04 (s, H_2 of **10a**), 8.12 (s, H_2), 8.35 (s, H_8), 8.73 (m, $CONHCH_2CH_2NHCO$); ^{13}C NMR δ 26.76 (CH_3), 38.40, 39.76 (CH_2CH_2), 72.70, 72.90 ($C_{2'}$, $C_{3'}$), 139.8 (C_8), 145.9 (C_2), 147.9 (C_4), 103.2 [N(NO)CONH], 156.5 (C_6), 169.64 (NHCO); MS, *m/e* 411 [(M + 1)$^+$], 410 (M$^+$). Anal. $C_{14}H_{18}N_8O_7 \cdot 0.5H_2O$) C, H, N.

1-Deoxy-1-(3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl)-*N*-[2-(3-methyl-3-nitrosoureido)ethyl]-β-D-ribofuranuronamide (9b). Compound **9b** was prepared from 1-deoxy-1-(3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl)-*N*-[2-(3-methylureido)-ethyl]-β-D-ribofuranuronamide (**6b**; 2.67 g, 7.4 mmol) as described above (preparation of **9a**). The reaction mixture was stirred for 1.5 h before evaporation to dryness in vacuo. An aqueous solution of the residue was deionized with Amberlite IR-120 (H) ion-exchange resin and then lyopholized. The residue crystallized from MeOH: yield 2.27 g (79%); mp 151–152 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) 256 nm at pH 1 (13.52), 256 at pH 7 (13.56), 262 at pH 13 (7.56); TLC $CHCl_3$–MeOH (3:1) (+5% acetic acid); ^{1}H NMR δ 2.84 (d, $NHCH_3$ of **10b**), 3.10 (s, CH_3), 3.37 (m, CH_2CH_2), 4.05 (m, $H_{3'}$), 4.17 (m, $H_{2'}$), 4.28 (d, $H_{4'}$), 4.6 (br, OH), 5.69 (d of d, H_5), 5.91 (d, $J_{1',2'}$ = 6 Hz, $H_{1'}$), 8.25 (d, H_6), 8.14 and 8.76 (2 m, $NHCH_2CH_2NH$), 11.35 (br s, N_3H); ^{13}C NMR δ 26.83 (CH_3), 72.83 ($C_{2'}$ and $C_{3'}$), 83.16 ($C_{4'}$), 87.8 ($C_{1'}$), 101.94 (C_5), 141.24 (C_6), 150.94 (C_2), 153.12 [N(NO)CONH], 163.02 (C_4), 170.18 (NHCO) (CH_2CH_2 covered by Me_2SO-d_6); MS, *m/e* 387 [(M + 1)$^+$]. Anal. ($C_{13}H_{18}N_6O_8$) C, H, N.

***N*-[2-(3-Cyclohexyl-1-nitrosoureido)ethyl]-1-deoxy-1-(1,6-dihydro-6-oxo-9*H*-purin-9-yl)-β-D-ribofuranuronamide (11a).** Nitrosation of **7a** (225 mg, 0.5 mmol) was carried out in formic acid as described for the methylureido compound **6a** and an aqueous solution of the residue was deionized with Amberlite IR-120 (H$^+$) ion-exchange resin and lyopholized. Purification of the product was carried out by preparative chromatography on Analtech avicel cellulose plates of 1000-μm thickness using H_2O as the developing solvent. The product was obtained by MeOH extraction. Evaporation of the MeOH solution gave a solid, which could not be crystallized from aqueous ethanol: yield 56 mg (22%); mp indefinite; TLC $CHCl_3$–MeOH–HOAc (17:3:1); UV λ_{max} ($\epsilon \times 10^{-3}$) 246 nm at pH 1 (16.14), 246 at pH 7 (15.94), 253 at pH 13 (13.58); ^{1}H NMR δ 1.1 (m, CH_3 of EtOH), 1.25 and 1.7 (2 m, CH_2 of C_6H_{11}), 3.25 (m, $CONHCH_2$), 3.5 (m, CH_2 of EtOH), 3.6 (br m, CH of C_6H_{11}), 3.92 [t, $CH_2N(NO)$], 4.15 (d, $H_{3'}$), 4.27 (s, $H_{4'}$), 4.5 (m, $H_{2'}$), 4.8 (br m, OH and H_2O), 5.95 (d, $J_{1',2'}$ = 6 Hz, $H_{1'}$), 8.05 (m, H_2), 8.37 (br m, $CONHCH$), 8.6 (H_8), 8.53 (m, $CONHCH_2$), 11.5 (br s, N_3H). Anal. ($C_{19}H_{26}N_8O_7 \cdot 0.6EtOH \cdot 0.5H_2O$) C, H, N.

***N*-[2-(3-Cyclohexyl-1-nitrosoureido)ethyl]-1-deoxy-1-(3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl)-β-D-ribofuranuronamide (11b).** Nitrosation of **7b** (100 mg, 0.24 mmol) was carried out as described for the methyureido compound **6a**. The material crystallized from MeOH in 38% yield: mp 144–145 °C dec; TLC *n*-BuOH–H_2O (6:1); UV λ_{max} ($\epsilon \times 10^{-3}$) 251 nm at pH 1 (13.35), 257 at pH 7 (13.31), 261 at pH 13 (7.84); ^{1}H NMR δ 1.35 and 1.75 (2 m, CH_2 of C_6H_{11}), 3.15 (m, $CONHCH_2$), 3.7 (br m, CH_2 of C_6H_{11}), 3.85 [t, $CH_2N(NO)$], 4.05 (m, $H_{3'}$), 4.1 (s, $H_{2'}$), 4.17 (m, $H_{4'}$), 4.4 (br, OH and H_2O), 5.7 (d of d, H_5), 5.9 (d, $J_{1',2'}$ = 6 Hz, $H_{1'}$), 8.2 (d, H_6), 8.3 (t, $CONHCH_2$), 8.38 (m,

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CONHCH), 11.33 (br s, N_3H); MS, m/e 454 (M^+). Anal. ($C_{18}H_{26}N_6O_8 \cdot 0.7H_2O$) C, H, N.

1,2-Dideoxy-1-(3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl)-*N*-[2-(3-methylureido)ethyl]-β-D-ribofuranuronamide (15). Compound **15** was prepared from 1,2-dideoxy-1-(3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl)-β-D-ribofuranuronic acid[26] (**14**; 1.28 g, 5 mmol) as described above for **6a**. The analytical sample was obtained by recrystallization from MeOH and then from H_2O: yield 1.44 g (77%); mp 233–235 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) 266 nm at pH 1 (9.44), 265 at pH 7 (9.47), 266 at pH 13 (7.02); TLC $CHCl_3$–MeOH (3:1); ^1H NMR δ 1.18 (m, CH_3 of Et_3N), 1.80 (s, 5-CH_3), 2.17 (m, 2 $H_{2'}$), 2.53 (d, CH_3NH), 3.12 (s, CH_2CH_2), 3.3 (br, H_2O), 4.21 (s, $H_{4'}$), 4.37 (br s, $H_{3'}$), 5.8 (m, CH_2NH), 6.32 (t, $J_{1',2'} = 8$ Hz, $H_{1'}$), 8.12 (H_6), 11.3 (br s, N_3 H); ^{13}C NMR δ 12.20 (5-CH_3), 26.32 (CH_3NH), 38.11, 38.91, and 39.78 ($C_{2'}$ and CH_2CH_2), 73.58 ($C_{3'}$), 85.27 and 85.46 ($C_{1'}$ and $C_{4'}$), 109.46 (C_5), 137.0 (C_6), 150.67 (C_2), 158.87 (NHCONH), 163.70 (C_4), 170.27 (NHCO); MS, m/e 356 [$(M + 1)^+$]. Anal. ($C_{14}H_{21}N_5O_6$) C, H, N.

***N*-[2-(3-Cyclohexylureido)ethyl]-1,2-dideoxy-1-(3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl)-β-D-ribofuranuronamide (16).** Compound **16** was prepared in 84% yield (724 mg) from **14** (512 mg, 12.00 mmol) as described above for **6a**: mp 242–244 °C; TLC $CHCl_3$–MeOH (3:1); UV λ_{max} ($\epsilon \times 10^{-3}$) 265 nm at pH 1 (9.24), 265 at pH 7 (9.35), 265 at pH 13 (7.07); ^1H NMR δ 1.15 and 1.65 (2 m, CH_2 of C_6H_{11}), 1.8 (s, CH_3), 2.12 (m, 2 $H_{2'}$), 3.11 (m, CH_2CH_2), 3.4 (br m, CH of C_6H_{11}, OH), 4.2 (s, $H_{4'}$), 4.38 (m, $H_{3'}$), 5.8 (m, NHCONH), 6.35 (t, $J_{1',2'} = 6$ Hz, $H_{1'}$), 8.12 (m, H_6), 8.22 (s, CONH); MS, m/e 423 (M^+), 424 [$(M + 1)^+$]; ^{13}C NMR δ 12.23 (CH_3), 24.48 (C_3 and C_5 of C_6H_{11}), 25.25 (C_4 of C_6H_{11}), 33.24 (C_2 and C_6 of C_6H_{11}), 47.81 (C_1 of C_6H_{11}), 73.60 ($C_{3'}$), 85.20 and 85.44 ($C_{1'}$ and $C_{4'}$), 109.46 (C_5), 136.92 (C_6), 150.67 (C_2), 157.54 (NHCONH), 163.68 ($C_{4'}$), 170.25 (NHCO). Anal. ($C_{19}H_{29}N_5O_2$) C, H; N: calcd, 16.53; found, 16.12.

1,2-Dideoxy-1-(3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl)-*N*-[2-(3-methyl-3-nitrosoureido)ethyl]-β-D-ribofuranuronamide (18). Nitrosation of **15** (940 mg, 2.53 mmol) was carried out as described above for **6a**. The product was purified by chromatography on Brinkman silica gel plates (2 mm) using $CHCl_3$–MeOH–HOAc as the developing solvent. The major band was eluted with MeOH. An equal volume of water was added and the solution treated with Amberlite IR-120 (H^+). Removal of the MeOH in vacuo caused crystallization: yield 378 mg (39%); mp 144–145 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) 262 nm at pH 1 (12.38), 262 at pH 7 (12.25), 266 at pH 13 (7.60); TLC $CHCl_3$–MeOH (5:1) (+5% acetic acid); ^1H NMR δ 1.78 (s, 5-CH_3), 2.1 (m, 2 $H_{1'}$), 2.84 (d, $NHCH_3$ of **19**), 3.09 [s, $CH_3N(NO)$], 3.37 (m, CH_2CH_2), 4.21 (m, $H_{4'}$ and $O_{3'}$ H), 4.36 (m, $H_{3'}$), 6.34 [t, $J_{1',2'} = 8$ Hz, $H_{1'}$), 8.08 (m, H_6), 8.35 and 8.78 ($CONHCH_2$ and NHCO); MS, m/e 384 (M^+) and 385 [$(M + 1)^+$]. Anal. ($C_{14}H_{20}N_6O_7$) C, H, N.

TLC of the filtrate indicated that it contained more product plus an isomeric nitrosourea. These isomers were separated on a thick plate and crystallized: yield of **18** 25 mg (total yield 41%). The yield of **19** was 15 mg (1.5%): ^1H NMR δ 1.8 (s, 5-CH_3), 2.1 (m, 2 H_2), 2.83 (d, $NHCH_3$), 3.15 (m, $NHCH_2$), 3.3 (s, H_2O), 3.9 [m, $N(NO)CH_2$], 4.12 (s, $H_{4'}$), 4.34 (m, $H_{3'}$), 0.56 (q, $CONHCH_3$), 11.28 (br, N_3H); MS (EI), m/e 282 [$(M - CH_3NHCONHO)^+$], 156 [$(sugar - CH_3NHCONNOH)^+$], 126 [$(B + H)^+$].

***N*-[2-(3-Cyclohexyl-1-nitrosoureido)ethyl]-1,2-dideoxy-1-(3,4-dideoxy-3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl)-β-D-ribofuranuronamide (20).** Nitrosation of **16** (2.03 g, 4.8 mmol) was carried out as described above for **6a**. The product was crystallized from MeOH: yield 1.26 g (58%); mp 144–146 °C dec; TLC $CHCl_3$–MeOH–HOAc (18:2:1); ^1H NMR δ 1.8 (s, CH_3), 2.07 (m, 2 $H_{2'}$), 3.2 (m, $NHCH_2$), 3.7 (br, CH of C_6H_{11}), 3.9 [m, $N(NO)CH_2$], 4.14 (s, $H_{4'}$), 4.35 (br, $H_{3'}$), 5.56 (d, O $H_{3'}$), 6.33 (t, $J_{1',2'} = 6$ Hz, $H_{1'}$), 8.03 (s, H_6), 8.2 (m, CH_2NHCO), 8.38 (d, CHNHCO), 11.3 (s, N_3H); MS, m/e 452 (M^+). Anal. ($C_{16}H_{28}N_6O_7$) C, H, N.

***N*-(2-Aminoethyl)-*N'*-methylurea (25) and Ethylenebis(methylurea) (27).** A solution of methyl isocyanate (5.7 g, 0.1 mol) in $CHCl_3$ (25 mL) was added over a 40-min period to a stirring solution of ethylenediamine (6.0 g, 0.1 mol) in $CHCl_3$ (150 mL). The resulting mixture was stirred for 2 h and chilled in an ice bath, and the bis compound was collected by filtration as a white solid: yield 5.33 g (61%); MS, m/e 100 [$(174 - NH_2CONHCH_3)^+$], 144 [$(174 - NHCH_3)^+$], 174 (M^+).

Evaporation of the filtrate gave the mono compound as a syrup: yield 3.5 g (30%); TLC BuOH–HOAc–H_2O (5:2:3); ^1H NMR δ ($CDCl_3$), 1.53 (s, NH_2), 2.73 (d, CH_3), 2.77 (m, CH_2NH_2), 3.2 (q, $NHCH_2$), 6.0 (m, br, NH's); ^{13}C NMR ($CDCl_3$) δ 26.81 (CH_3), 42.45 (CH_2NH_2), 43.29 ($NHCH_2$), 160.50 (C=O); MS, m/e 88 [$(117 - NHCH_3 + H)^+$], 101 [$(117 - NH_2)^+$], 118 [$(M + 1)^+$].

An analytical sample of the monosubstituted compound was obtained as the picrate salt, mp 158–162 °C. Anal. ($C_{10}H_{14}N_6O_8 \cdot 0.5H_2O$) C, H, N.

***N*-(2-Aminoethyl)-*N*-cyclohexylurea (26) and Ethylenebis(cyclohexylurea) (28).** The reaction was carried out as described for **25** and **27**. The bis compound was obtained as a solid in 24% yield: MS, m/e 212 [$(310 - NHC_6H_{11})^+$], 229 [$(310 - C_6H_{11} + 2H)^+$], 310 (M^+).

The mono compound, obtained from the filtrate as a syrup that crystallized on standing, was recrystallized from benzene–hexane in 55% yield: mp 52–55 °C; TLC BuOH–HOAc–H_2O (5:2:3); ^1H NMR ($COCl_3$) δ, 1.53 (br m, cyclohexyl C-2 to C-6 protons), 1.84 (s, NH_2), 2.79 (t, CH_2NH_2), 3.23 (t, $NHCH_2$), 3.5 (br s, cyclohexyl C-1 proton), 5.32 (d, NHCO), 5.65 (t, CONH); ^{13}C NMR ($CDCl_3$) δ 25.01 (C-3 and C-5 of cyclohexyl), 25.74 (C-4 of cyclohexyl), 33.96 (C-2 and C-6 of cyclohexyl), 42.09 and 42.99 (CH_2CH_2), 48.98 (C-1 of cyclohexyl), 158.53 (NHCONH); MS, m/e 168 [$(185 - NH_3)^+$], 185 (M^+), 186 [$(M + 1)^+$].

An analytical sample of the mono compound was obtained as the picrate salt, mp 152–153 °C. Anal. ($C_{15}H_{22}N_6O_8$) C, H, N.

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