A New Route to ^{15}N-Labeled, *N*-Alkyl, and *N*-Amino Nucleosides via N-Nitration of Uridines and Inosines

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Received November 9, 1994®

Abstract: A novel method for the specific [3-^{15}N]-labeling of pyrimidine nucleosides and [1-^{15}N]-labeling of purine nucleosides is reported, according to Scheme 1. The N-nitration reaction is carried out in good yields with nitronium trifluoroacetate in cold dichloromethane. Treatment of the resulting *N*-nitro nucleosides with $^{15}NH_3$, alkylamines, or hydrazine cleaves the pyrimidine ring at room temperature, affording open intermediates which undergo cyclization to ^{15}N-labeled, N-alkylated, or *N*-amino nucleosides, respectively. Preparation of [1-^{15}N]adenosine from inosine in a 52% overall yield is illustrative of the scope of the procedure. [3-^{15}N,$^{15}NH_2$]-5′-*O*-Acetyl-3-amino-2′,3′-*O*-isopropylideneuridine and [1-^{15}N,$^{15}NH_2$]-2′,3′,5′-tri-*O*-acetyl-1-aminoinosine have also been obtained from double labeled hydrazine. By using a ^{15}N-labeled substrate and/or ^{15}N-labeled benzylamine it is shown that the amine attack takes mainly place at C4 of uridine and at C2 of inosine.

Introduction

Nucleosides specifically labeled with ^{15}N at the sites involved in hydrogen bonding enjoy several interesting applications: (i) investigation of possible hydrogen bonds among nucleosides (Watson−Crick and/or Hoogsteen patterns of base pairing) or between these nucleosides and other molecules or macromolecules (molecular recognition phenomena, in general); (ii) incorporation into nucleic acid fragments and elucidation of their structure; and (iii) study of protein−DNA and related interactions.[1] These applications are based on the fact that the changes undergone by the corresponding ^{15}N−H bonds can be readily and clearly monitored by ^1H and/or ^{15}N NMR spectroscopy.[2] Multiple quantum NMR techniques have also been successfully applied on multilabeled oligonucleotides to provide three-dimensional structural information.[1b,f,h]

Obviously, the relevant sites for ^{15}N labeling are (i) the amino groups of cytidine, adenosine, and guanosine derivatives, (ii) N3 of pyrimidine nucleosides, and (iii) N1 and N7 of purine nucleosides. The first possibility is the most simple route to labeled nucleosides and is achieved by conversion of uridine to C4-activated derivatives,[3a,c] followed by treatment with labeled ammonia to afford [4-$^{15}NH_2$]cytidines, and by conversion of appropriate purine derivatives[3b,c] into [6-$^{15}NH_2$]adenosines or [2-$^{15}NH_2$]guanosines.[4] Concerning [3-^{15}N]pyrimidine nucleosides, the work of Lawson and DeGraw,[5a] who synthesized multilabeled thymidines from [$^{15}N_2$]urea and labeled 2-cyanopropanoic acid, that of Poulter and Livingston,[2d] on a total synthesis of [3-^{15}N]-2′,3′,5′-tri-*O*-benzoyluridine from 3-aminopropanoic acid and KOC^{15}N, and that of Niu,[5b] who prepared $^{15}N_2$-enriched uracil from [$^{15}N_2$]urea and propynoic acid and then converted it to double labeled uridines and cytidines, are remarkable. With regard to the N1- or N7-labeling of purine nucleosides, the approaches reported so far could be classified into two groups: the "synthetic" one, in which either the azole or azine ring is built upon the other one with incorporation of the label at a suitable step,[6] and that based on some type of rearrangement of the purine system (generally through a ring-opening/ring-closing mechanism).[7]

In this connection, we report on *a novel procedure for the [3-^{15}N]-labeling of pyrimidine nucleosides and [1-^{15}N]-labeling*

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® Abstract published in *Advance ACS Abstracts*, March 1, 1995.

(1) (a) Gaffney, B. L.; Goswami, B.; Jones, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 12607. (b) Sklenár, V.; Peterson, R. D.; Rejante, M. R.; Wang, E.; Feigon, J. *J. Am. Chem. Soc.* **1993**, *115*, 12181, and references therein. (c) Rhee, Y.; Wang, C.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 8742, and references therein. (d) Goswami, B.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 3832. (e) Gaffney, B. L.; Wang, C.; Jones, R. A. *J. Am. Chem. Soc.* **1992**, *114*, 4047. (f) Wang, C.; Gao, H.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1991**, *113*, 5486. (g) Massefski, W.; Redfield, A.; Sarma, U. D.; Bannerji, A.; Roy, S. *J. Am. Chem. Soc.* **1990**, *112*, 5350. (h) Davis, D. R.; Yamaizumi, Z.; Nishimura, S.; Poulter, C. D. *Biochemistry* **1989**, *28*, 4105, and references therein. (i) Gao, X.; Jones, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 3169. (j) Kupferschmitt, G.; Schmidt, J.; Schmidt, T.; Fera, B.; Buck, F.; Rüterjans, H. *Nucleic Acid Res.* **1987**, *15*, 6225. (k) Roy, S.; Papastavros, M. Z.; Sanchez, V.; Redfield, A. G. *Biochemistry* **1984**, *23*, 4395.

(2) For pioneering works, see: (a) Katz, L.; Pennan, J. *J. Mol. Biol.* **1966**, *15*, 220. (b) Shoup, R. R.; Miles, H. T.; Becker, E. D. *Biochem. Biophys. Res. Commun.* **1966**, *23*, 194. (c) Scheit, K. H. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 180. (d) Poulter, C. D.; Livingston, C. L. *Tetrahedron Lett.* **1979**, 755, and references therein. (e) Kanamori, K.; Roberts, J. D. *Acc. Chem. Res.* **1983**, *16*, 35, and references therein.

(3) (a) Ueda, T. *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, 1988; Vol. 1, p 1. (b) Srivastava, P. C.; Robins, R. K.; Meyer, R. B. *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, 1988; Vol. 1, p 113. (c) Vorbrüggen, H. *Adv. Heterocycl. Chem.* **1990**, *49*, 117.

(4) (a) Fera, B.; Singrün, B.; Kupferschmitt, G.; Schmidt, J.; Buck, F.; Rüterjans, H. *Nucleosides Nucleotides* **1987**, *6*, 477. (b) Kieper, I.; Schmidt, T.; Fera, B.; Rüterjans, H. *Nucleosides Nucleotides* **1988**, *7*, 821. (c) Sarfati, S. R.; Pochet, S. *J. Labelled Compd. Radiopharm.* **1991**, *29*, 1323. (d) For the synthesis of [4-^{15}N]cytidine and [6-^{15}N]adenosine by using [^{15}N]phthalimide instead of $^{15}NH_3$, see: Kamaike, K.; Takahashi, M.; Utsugi, K.; Tomizuka, K.; Ishido, Y. *Tetrahedron Lett.* **1995**, *36*, 91.

(5) (a) Lawson, J. A.; DeGraw, J. I. *Nucleic Acid Chemistry*; Townsend, L. B., Tipson, S., Eds.; Wiley: New York, 1978; Part 2, p 921. (b) Niu, C.-H. *Anal. Biochem.* **1984**, *139*, 404. For previous preparations of [1,3-$^{15}N_2$]uracil, see: Harada, K.; Suzuki, S. *Tetrahedron Lett.* **1976**, 2321. Lipnick, R. L.; Fissekis, J. D. *J. Labelled Compd. Radiopharm.* **1979**, *17*, 247.

(6) (a) Gaffney, B. L.; Kung, P.-P.; Jones, R. A. *J. Am. Chem. Soc.* **1990**, *112*, 6748 ([7-^{15}N]deoxyadenosine and [7-^{15}N]deoxyguanosine from 4,6-diaminopyrimidine and 2,4,6-triaminopyrimidine, respectively, by using $^{15}N_b$-labeled benzenediazonium salt). (b) Reference 1f ([7-^{15}N]guanosine from 2,6-diamino-4-hydroxypyrimidine and $Na^{15}NO_2/H^+$). (c) Golding, B. T.; Slaich, P. K.; Watson, W. P. *J. Chem. Soc., Chem. Commun.* **1986**, 901 ([1-^{15}N]guanosine from AICA-riboside and $^{15}NH_3$). (d) Bleasdale, C.; Ellwood, S. B.; Golding, B. T.; Slaich, P. K.; Taylor, O. J.; Watson, W. P. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2859 ([1-^{15}N]guanosine and [1-^{15}N]-deoxyguanosine from AICA-riboside). (e) For a related work, see: Bhat, B.; Groziak, M. P.; Leonard, N. J. *J. Am. Chem. Soc.* **1990**, *112*, 4891.

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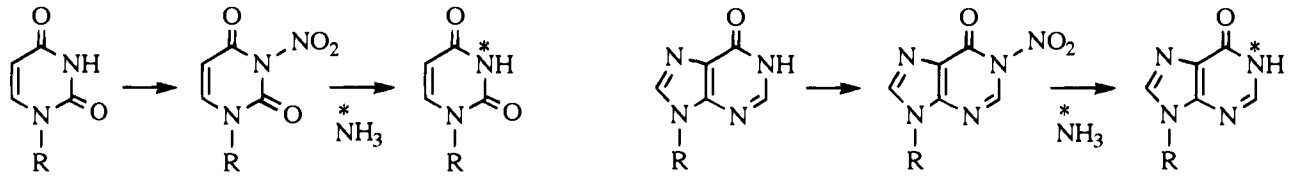
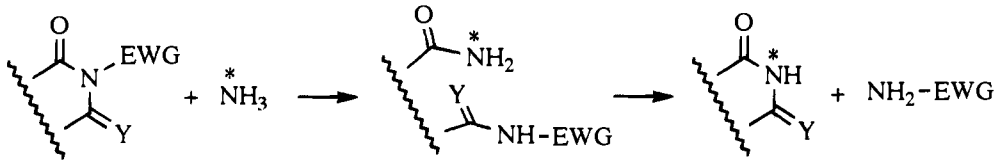
Scheme 1



Scheme 2



of purine nucleosides (see Scheme 1), which avoids carrying out de novo syntheses for each different substrate and which in principle could be applied to the final product of any nucleoside or nucleotide synthesis, because it relies on a simple ring-opening/ring-closing process that should occur under mild conditions. The underlying idea may be summarized as shown in Scheme 2.

Provided that a strong electron-withdrawing group (EWG) could be bound to the selected nitrogen of an imide-like substructure, ammonia could be active enough, even at room temperature, to attack the substrate at a vicinal electrophilic carbon atom. Though the carboxamide group of the expected intermediate—only one of the two possible intermediates is depicted in Scheme 2 for the sake of simplicity—is a poor nucleophile, the cyclization to give a six-membered ring may compensate this handicap. Moreover, the nature of the leaving group, NH_2EWG or $NH(EWG)^-$, may be of help in the cyclization steps. Apparently, taking everything into account the first choice should be a NO_2 group: only N_2^+, SMe_2^+, NR_3^+, and SO_2CF_3 are better EWG than NO_2 among the usual organic substituents,[8] but the first three cannot be directly introduced at the selected nitrogen and the last one would prefer to bind to the carbonyl oxygen atom by triflate formation rather than to the nitrogen atom;[3a,b] furthermore, HN_3, $HNSMe_2$, and $HNNR_3$ are not appropriate leaving groups, in contrast to $HNNO_2^-$, or nitramine anion, which readily decomposes to give N_2O.[9] Thus, in order to check the feasibility of the method it was essential to investigate first whether the conversion of pyrimidine and purine nucleosides to the corresponding *N*-nitro derivatives was possible or not. Reaction of these *N*-nitro compounds with $^{15}NH_3$ was then studied. Since other nucleophiles may also attack the C=O or C=Y groups drawn in Scheme 2, the reaction of amines and hydrazine with substrates such as those illustrated above will also be described here to complement the data arising from the reactions with $^{15}NH_3$.

Results and Discussion

N-Nitration of Uridines. To achieve a mild N-nitration reaction, avoiding the nucleoside hydrolysis and loss of the sugar-ring substituents and protecting groups, was the first challenge. It is well-known that by treatment with HNO_3 nucleosides may undergo O-nitration, oxidation of primary alcohols to acids, and/or nitration at C5 (in the uridine case), depending on the reaction conditions and protecting groups,[3] but *no N-nitro nucleoside has been reported to the best of our knowledge*. Preliminary experiments on 2′,3′,5′-tri-*O*-acetyluridine (**1a**), using either (i) nitronium benzoate (prepared from $AgNO_3$/PhCOCl in CH_3CN and filtering off the precipitate of AgCl),[10] (ii) $Cu(NO_3)_2/Ac_2O$,[11] or (iii) NO_2BF_4 in CH_3CN, at different temperatures, with or without 2,6-di-*tert*-butyl-4-methylpyridine,[12] were unsuccessful, since starting material was mostly recovered. On the other hand, NO_2OCOCF_3 (nitronium trifluoroacetate or nitric trifluoroacetic anhydride), under appropriate conditions,[12] gave very good results. In fact, when *completely O-protected uridines* **1a** and **1b** were treated with this mixed anhydride, generated from NH_4NO_3 (2 equiv) and $(CF_3CO)_2O$ (4 equiv), excellent yields of the desired N-nitro derivatives, **2a** and **2b**, were respectively obtained (Scheme 3). Identical treatment of *partially protected uridine* **1c** afforded mixtures of the *O*-nitro compound (nitrate ester) and 2′,3′-*O*-isopropylidene-3,5′-*O*-dinitrouridine (**2c**), indicating that nitration of primary hydroxyl group was more rapid than that of the imide-like NH; with a larger excess of the nitrating mixture, the *N,O*-dinitro compound **2c** was formed in quantitative yields. In the case of tribenzoyl derivative **1d**, which was more recalcitrant to N-nitration, a larger excess of reagents had to be employed for improving the yield (from 70% to 80%), although 20% of starting material still remained in the crude product.

As shown in Scheme 3, the $N-NO_2$ group is stable in acidic media, since the alcohol-protecting groups could be removed by treatment with MeOH/HCl at room temperature[13] to afford 3-nitrouridine (**2**) in ca. 80% isolated yields.

Disappearance of the NH proton, small shifts to downfield of the H5 and H6 protons, and large shifts to upfield of C2

(7) (a) De Napoli, L.; Messere, A.; Montesarchio, D.; Picialli, G.; Santacroce, C.; Varra, M. *J. Chem. Soc., Perkin Trans. 1* **1994**, 923 ([1-^{15}N]deoxyinosine from a dimeric purine and aqueous $^{15}NH_3$). (b) Goswami, B.; Jones, R. A. *J. Am. Chem. Soc.* **1991**, *113*, 644 ([1-^{15}N]deoxyguanosine from [6-$^{15}NH_2$]deoxyadenosine via N-oxidation, treatment with BrCN, ring opening, etc.). (c) Adler, J.; Powell, W.; Wolfenden, R. *J. Am. Chem. Soc.* **1990**, *112*, 1247 ([1-^{15}N]purine ribonucleoside from 1-methylpurinium ribonucleoside cation with $^{15}NH_3$). (d) Gao, X.; Jones, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 1275 ([1-^{15}N] and [6-$^{15}NH_2$]deoxyadenosine from deoxyadenosine, by replacement of Cl and Dimroth-like rearrangement), and references therein. For related papers, see: (e) Kos, N. J.; Jongejan, J.; van der Plas, H. C. *Gazz. Chim. Ital.* **1987**, *117*, 369. (f) Barrio, M. C. G.; Scopes, D. I. C.; Holtwick, J. B.; Leonard, N. J. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3986.

(8) Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91*, 165.

(9) For recent studies on nitramide, see: (a) Attinà, M.; Cacace, F.; Ciliberto, E.; Petris, G.; Grandinetti, F.; Pepi, F.; Ricci, A. *J. Am. Chem. Soc.* **1993**, *115*, 12398. (b) Ritchie, J. P. *J. Am. Chem. Soc.* **1989**, *111*, 2517.

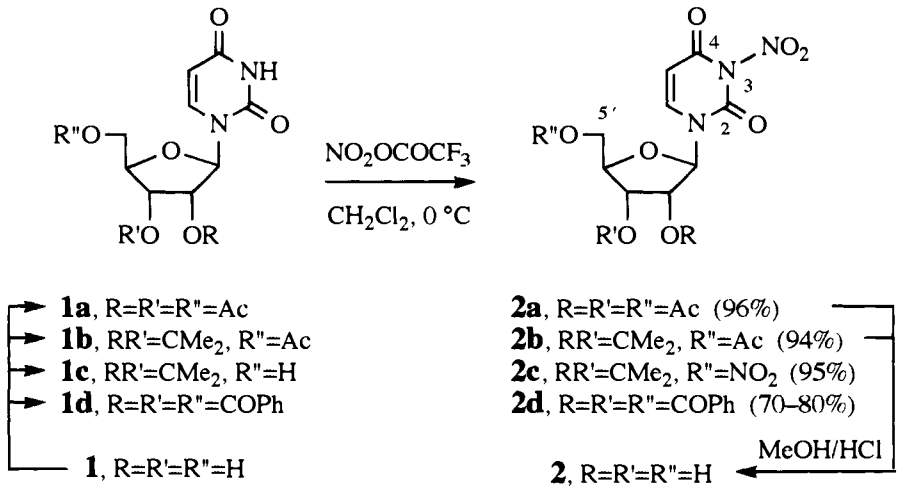
(10) (a) Kurz, M. E.; Yang, L. T. A.; Zahora, E. P.; Adams, R. C. *J. Org. Chem.* **1973**, *38*, 2271. (b) The best N-nitration yield with this reagent (2 equiv) was 16% of **2a** after 24 h at 65 °C.

(11) (a) Davey, W.; Gwitt, J. R. *J. Chem. Soc.* **1950**, 204. (b) Campbell, R.; Peterson, C. J. *J. Org. Chem.* **1963**, *28*, 2294.

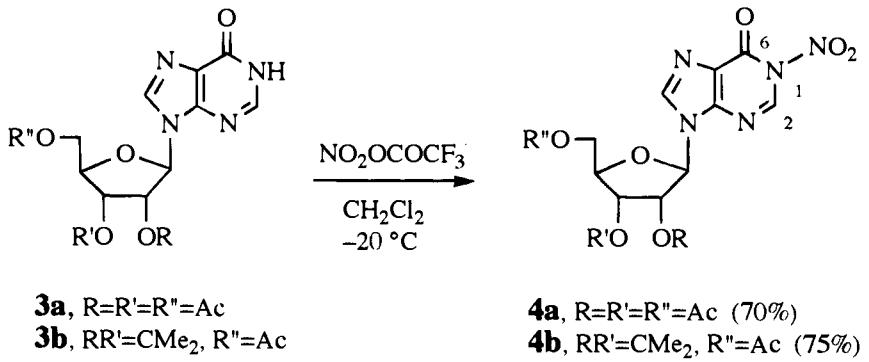
(12) Romea, P.; Aragonès, M.; Garcia, J.; Vilarrasa, J. *J. Org. Chem.* **1991**, *56*, 7038, and references therein.

(13) Under these conditions, the $N-NO_2$ and $O-NO_2$ bonds are not cleaved.

Scheme 3



Scheme 4



($\Delta\delta \approx -5$ ppm) and C4 ($\Delta\delta \approx -8$ ppm)[14] are the main features of the NMR spectra of *N*-nitro uridines in relation to their parent compounds. Dinitrouridine **2c**, in addition, showed relatively large δ values for its H5′/H5″ and C5′ nuclei (δ 4.71 and δ 71.8, respectively), as expected for a methylene of a nitrate ester.

While esters and acetals were stable under the nitration conditions (i.e., not only in the presence of the nitrating agent but also of CF_3COOH, a reaction product[12]), this was not the case of the triphenylmethyl, benzyl, and *tert*-butyldimethylsilyl ethers. In fact, complex mixtures of products nitrated at the aromatic rings were observed in the case of 2′,3′-*O*-isopropylidene-5′-*O*-(triphenylmethyl)uridine and 2′,3′,5′-tri-*O*-benzyluridine. Silyl groups were partially removed to give mixtures of nitrate and trifluoroacetate esters.

N-Nitration of Inosines. In the light of the results just described, only purine nucleosides with hydroxyls protected as esters or acetals were considered. When 2′,3′,5′-tri-*O*-acetylinosine (**3a**) was treated with NO_2OCOCF_3 under the above-mentioned conditions, nitration took place readily to afford its 1-nitro derivative (**4a**) but also a significant amount of decomposition products. Fortunately, yields of isolated **4a** increased by carrying out the reaction at lower temperature (Scheme 4). Other possible nitrating mixtures, which should give rise to a less acidic medium than the $NH_4NO_3/(CF_3CO)_2O$ mixture, such as nitronium benzoate, nitronium 3,5-dinitrobenzoate, $Cu(NO_3)_2/Ac_2O$, or NO_2BF_4/2,6-di-*tert*-butyl-4-methylpyridine, did not improve the yields; as in the uridine case, starting material was mostly recovered. Nitration of 5′-*O*-acetyl-2′,3′-*O*-isopropylideneinosine, **3b**, with NO_2OCOCF_3 gave its 1-nitro derivative (**4b**) in good yield as well.

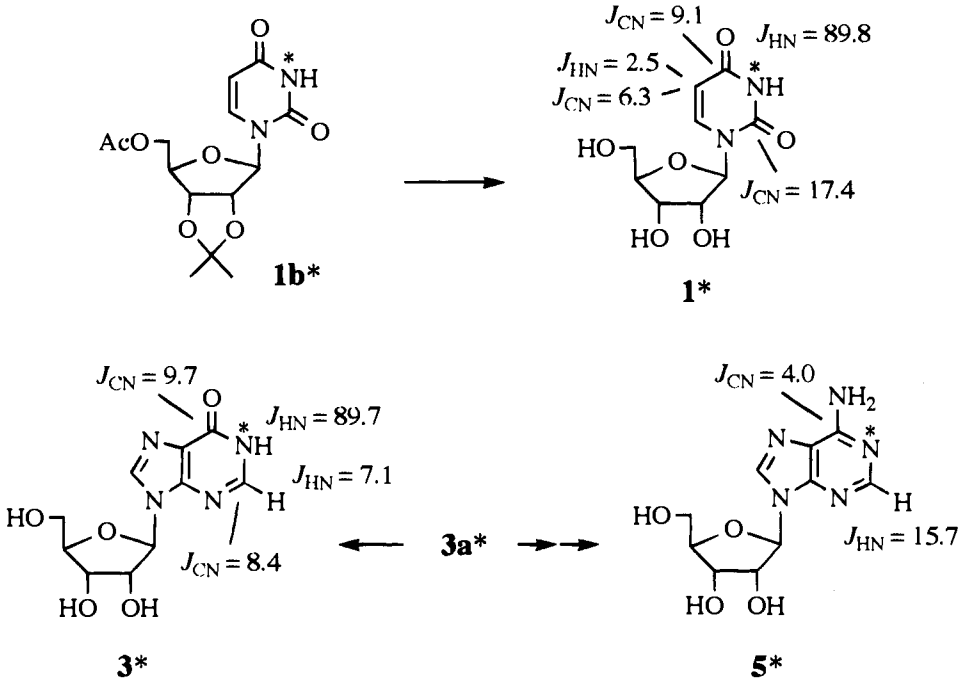
(14) We observed a similar effect on the δ(CO) for five- to eight-membered lactams: Torra, N.; Urpí, F.; Vilarrasa, J. *Tetrahedron* **1989**, *45*, 863.

It deserves comment that tri-O-acetylated N^6-benzoyladenosine did not react with NO_2OCOCF_3, even with a large excess of reagent, and that tri-O-acetylated guanosines, such as 2-amino, 2-benzamido, and 2-[(dimethylamino)methylene]amino derivatives of **3a**, were unsuitable because under treatment with NO_2OCOCF_3 did not react (benzoyl derivatives) or decompose. 2′-Deoxyinosine derivatives were N-nitrated but they turned out to be too sensitive to the medium, as cleavage of the glycoside bond was observed. These are intrinsic limitations of the method in its present status, since it can be successfully applied only to lactam-like and/or imide-like structures with sugar moieties stable in the reaction medium.

The nitro group of nitroinosines **4a** and **4b**, as compared to **3a** and **3b**, shifts the H2 proton 0.5 ppm downfield and its C2 and C6 vicinal carbons ca. 5 and ca. 14 ppm, respectively, upfield. As good crystals of **4a** could be obtained (from MeOH), this compound was submitted to X-ray diffraction analysis. The crystal was very sensitive to X-rays—a rather rapid decay in the intensity was observed—but the structure could be solved (see supplementary material) confirming that the nitro group was on N1.

Reaction of *N*-Nitro Nucleosides with $^{15}NH_3$. When a solution of 5′-*O*-acetyl-2′,3′-*O*-isopropylidene-3-nitrouridine (**2b**) was added to a mixture of $^{15}NH_4Cl$ (only 1.2 equiv), KOH, and Et_3N in CH_3CN-H_2O at room temperature, a smooth reaction took place to give a much more polar product. A few days later, this product had been almost completely converted into a new compound that was chromatographically and spectroscopically identical to 5′-*O*-acetyl-2′,3′-*O*-isopropylideneuridine (**1b**), except for the appearance of $^1H-^{15}N$ and $^{13}C-^{15}N$ couplings in its ^1H and ^{13}C NMR spectra. The isolated

Scheme 5



yield of the labeled compound (**1b***) was 73%.[15a] Its proton-coupled ^{15}N NMR spectrum showed the expected double doublet ($^1J_{NH}$ = 91.0 Hz, $^3J_{N,5}$ = 2.7 Hz) at δ −210.6.[2e] Deprotection of **1b*** with MeOH/HCl gave [3-^{15}N]uridine (**1***) in excellent yield.

In an alternative experiment, **2b** was treated with $^{15}NH_4Cl$ (1 equiv) and K_2CO_3 (2 mmol per mmol) in CH_3CN-H_2O at room temperature for 4 days, to afford **1b*** in 65% isolated yield.[15b]

N-Nitro inosine **4a** was more reactive than *N*-nitro uridines. By treatment with $^{15}NH_3$ at room temperature, a 85% yield of [1-^{15}N]-2′,3′,5′-tri-*O*-acetylinosine (**3a***) was achieved within few hours. This compound could be readily deprotected to [1-^{15}N]inosine (**3***, see Scheme 5) with either concentrated NH_3 in MeOH or with MeOH/HCl. It could also be transformed to [1-^{15}N]adenosine (**5***) in 87% overall yield by means of a known sequence (treatment with $SOCl_2$/DMF in CH_2Cl_2 followed by heating with NH_3/MeOH in a sealed tube).[16] Scrambling or loss of label was not observed.

Reaction of *N*-Nitro Nucleosides with Alkyl Amines. With regard to nitrogen nucleophiles other than ammonia, it is noteworthy that treatment of nitrouridine **2a** with an excess of 33% ethanolic methylamine, at room temperature, afforded *N*-methyluridine in 70% yield after purification by column chromatography; thus, the expected substitution of NMe for NNO_2 occurred with concomitant deacetylation, caused by the excess of methylamine.

Butylamine and benzylamine also reacted with nitrouridines. For example, polar products were mostly formed from **2b** at room temperature after a few hours of reaction in CH_3CN, by using 2 equiv of these amines (or 1 equiv plus Et_3N or K_2CO_3), but these intermediate products did not spontaneously give cyclic compounds. To force the conversion into *N*-butyl- and *N*-benzyluridines we investigated both the temperature effect and the role of the medium acidity or basicity. Whereas addition of CF_3COOH gave rise to decomposition and the appearance of many byproducts, addition of K_2CO_3 and heating at 70 °C appeared to be the conditions of choice. An excess of butylamine or benzylamine was detrimental as several byproducts were formed, including open species with a *trans* C−C double bond (isomerization taking probably place via a conjugate addition-elimination). Moreover, the reaction did not work with amines like isopropylamine, due to a very low rate of attack to **2b** and the scarce stability of the intermediates. Reaction of **2b** with methyl glycinate was not satisfactory: while nothing occurred at room temperature, a 22% yield of the N^3-CH_2-COOMe derivative (42% on the consumed starting material) was achieved with methyl glycinate hydrochloride and K_2CO_3 in refluxing CH_3CN for 1 h, but attempts of improving the yield either by using an excess of amino ester or by lengthening the reaction time were detrimental, as a large number of byproducts were formed. Reaction of **2b** with glycine and K_2CO_3 was unsuccessful as well.

The reaction of **2b** with benzylamine was investigated in more detail. When **2b** was treated with 2 equiv of benzylamine, in $CDCl_3$ at room temperature, only one polar intermediate was obtained, which was kinetically stable under these conditions. The H5 and H6 protons of **2b**, at δ 5.87 and 7.40, were shifted to δ 5.49 and 6.25, which is obviously a consequence of the opening of the uracil ring; $J_{5,6}$ also changed from 8.3 Hz to 9.0 Hz. Although there is no doubt, in the light of the results reported throughout this work, that ring-opening/ring-closing processes operate,[17] we were interested in determining the structure of this intermediate in order to elucidate whether the initial attack took place chiefly at C4 or C2. We carried out two independent, additional experiments:

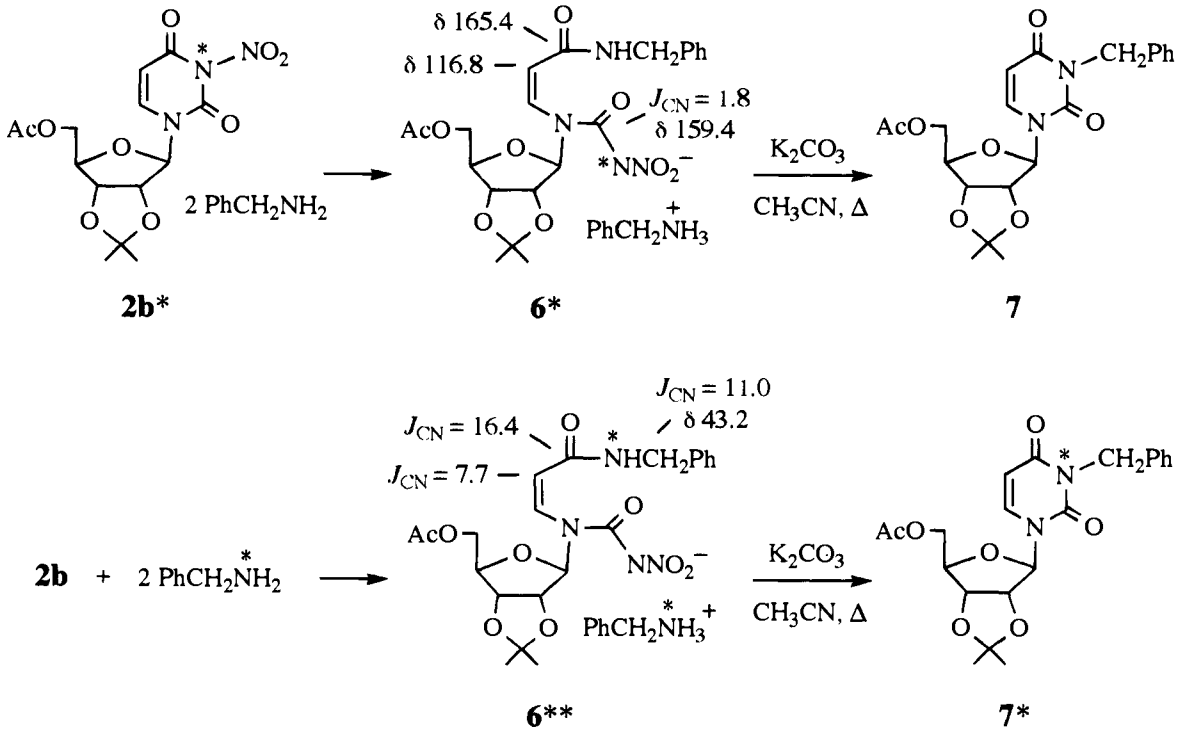
(i) A small amount of **1b*** was converted into [3-^{15}N]-labeled nitro derivative **2b***, which was treated with 2 equiv of benzylamine in $CDCl_3$. The different $^{13}C-^{15}N$ couplings (and the coupling between H5 and ^{15}N) noted in the *N*-nitro derivative disappeared, except for the carbon of the polar intermediate related to C2 of the uridine,[18] which was the only signal showing splitting (see **6***, Scheme 6). As the label is bound to the C2-related carbon atom, *the amine attack had to occur at C4.*

(ii) Reaction of **2b** with $PhCH_2{}^{15}NH_2$ (prepared by reduction of $PhCO^{15}NH_2$) was also monitored by NMR. The resulting

(15) (a) A small amount (4%) of the deacetylated derivative (**1c***) was also isolated. (b) Plus the deacetylated derivative (**1c***) in 11% yield.

(16) (a) Zemlicka, J.; Sorm, F. *Coll. Czech. Chem. Commun.* **1965**, *30*, 1880. (b) Robins, M. J.; Basom, G. L. *Can. J. Chem.* **1973**, *51*, 3161.

Scheme 6



intermediate had the structure shown in Scheme 6 (**6****). *Thus, we confirmed that the attack of benzylamine on 3-nitrouridines takes place at C4.*

To achieve the cyclization of **6**/**6***/**6**** to 2′,3′,5′-tri-*O*-acetyl-*N*-benzyluridine or its ^{15}N-labeled counterpart (**7**/**7***) in good yield it was necessary to heat the former in CH_3CN in the presence of K_2CO_3, as already mentioned.

By contrast, 1-nitroinosine **4a** reacted quickly with alkylamines, since even at −78 °C either 2 equiv of methylamine or 1 equiv of methylamine plus 1 equiv of Et_3N were sufficient to cleave the six-membered ring of the purine system; conversion to the corresponding 1-methylinosine derivative (see **8**, R = Me) proceeded smoothly at room temperature. Butylamine and benzylamine also reacted with nitroinosines **4a** and **4b** more quickly than with nitrouridines **2a** and **2b**; subsequent ring closing of the polar intermediates was studied, as in the uridine case, at different temperatures and in the presence of either CF_3COOH, K_2CO_3, or an excess of butylamine or benzylamine. Addition of CF_3COOH was beneficial since the reaction times for obtaining the desired 1-butyl- and 1-benzylinosines were shortened, whereas addition of K_2CO_3 was detrimental. We also noted that the reaction of **4a** with an excess of benzylamine in refluxing $CHCl_3$ gave a bis(benzylamino) derivative as the major product. This means that in nitroinosines the first ring-open intermediate is amenable either to cyclization or to a further attack of another amine molecule.

That the polar intermediates arising from nitroinosines were much more able to cyclize to *N*-alkyl inosines (as compared to the nitrouridine case) was made evident by treating **4a** with bulkier and/or poorer nucleophilic amines such as isopropylamine, methyl glycinate, and methyl L-alaninate, which afforded the corresponding N-substituted inosines (see **8**) in good yield. Even *tert*-butylamine, methyl L-valinate, and methyl L-isoleucinate gave the corresponding reaction, though in lower yield. Since no epimerization at the N-CHR-COOMe stereocenter was noted in the last case (**8**, R = CH(sBu)COOMe, with the *S,S* configuration) under the described conditions, it can be stated that the reactions involving chiral amines take place with configuration retention, as could be expected at first sight.

The attack of nitroinosines by benzylamine was also studied in more detail by using ^{15}N-labeled benzylamine. When nitroinosine **4a** was allowed to react with $PhCH_2{}^{15}NH_2$ in $CDCl_3$ at room temperature, the corresponding intermediate showed the H2-related proton to be coupled with the ^{15}N label (δ 8.49, $^2J_{HN}$ = 7.5 Hz, $^3J_{HH}$ = 3.6 Hz). The C2-related carbon atom appeared as a doublet ($^1J_{CN}$ = 18.3 Hz), whereas the C6-related carbon did not show any splitting (see **9****, Scheme 7). Therefore, *the attack of benzylamine* (and presumably that of other amines) *on 1-nitroinosines takes place at C2.* According to what was observed in the reaction of **4a** with benzylamine, we confirmed that **9**** may follow two pathways, giving either **8*** (R = CH_2Ph) or, in the presence of an excess of ^{15}N-labeled benzylamine, the bis(benzylamino)-containing derivative **10****.

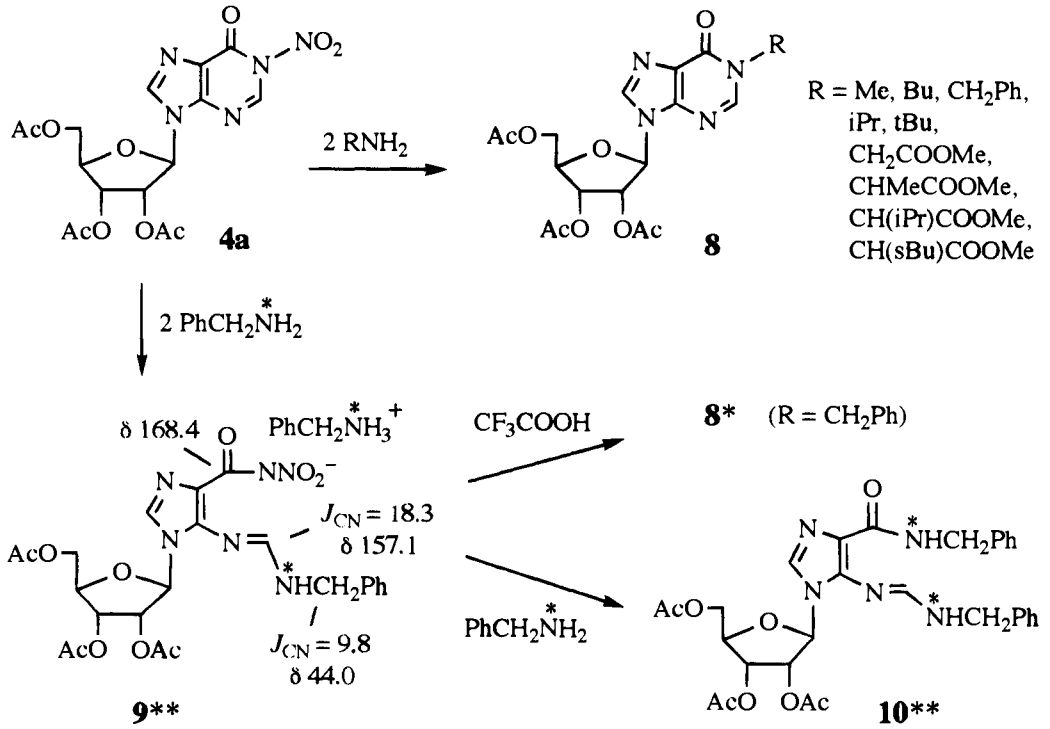
Formally, most of the reactions described in this section are equivalent to simple N-alkylations of uridines and inosines. Reactions with the simplest amines are actually devoid of practical interest, since there are known protocols for the selective alkylation of N3 of pyrimidine nucleosides[3a] and of N1 of purine nucleosides.[3b] However, selective incorporation of sterically crowded chains and/or chiral substituents on N1 of certain purine nucleosides, readily feasible by the method here reported, may find application in the near future.

Reaction of *N*-Nitro Nucleosides with Hydrazine. *N*-Nitro nucleosides reacted much more rapidly with hydrazine than with amines, as could be expected. For example, reaction of **2b** with an equivalent amount of hydrazine hydrate in MeOH gave 5′-*O*-acetyl-3-amino-2′,3′-*O*-isopropylideneuridine (**11**) almost quantitatively within 2 h; evolution of gas (presumably N_2O) was observed.

Even though *N*-aminouridines can be obtained from uridines by treatment of their sodium salts with 2,4-dinitrophenoxyamine in DMF for some days,[3a,19] and even though 1-aminoinosine and 1-aminoguanosine can be obtained from the parent compounds and hydroxylamine-*O*-sulfonic acid,[3b,20] the *indirect*

(17) For references on ANRORC-like mechanisms, see: (a) van der Plas, H. C. *Tetrahedron* **1985**, *41*, 237. (b) Hirota, K.; Kitade, Y.; Sajiki, H.; Maki, Y.; Yogo, M. *J. Chem. Soc., Perkin Trans 1* **1990**, 367. (c) Suwinski, J.; Szczepankiewicz, W. *Tetrahedron: Asymmetry* **1991**, *2*, 941.

Scheme 7



amination method reported here appears to be an elegant, alternative procedure in some special cases. An advantage of this approach is the possibility of label introduction inside the ring. Thus, from **2b**, commercially available [$^{15}N_2$]hydrazinium hydrogensulfate, and KOH in MeOH/H_2O, double labeled 5′-*O*-acetyl-3-amino-2′,3′-*O*-isopropylideneuridine (**11****) was readily obtained, in 85% yield. Likewise, we have prepared [1-^{15}N,$^{15}NH_2$]-2′,3′,5′-tri-*O*-acetyl-1-aminoinosine (**12****) by expending only 1 mol per mol of the double labeled hydrazinium sulfate.

Both the ^{1}H and ^{15}N NMR spectra indicated clearly that a six-membered *N*-amino derivative (i.e., the N-NH_2 arrangement) was obtained rather than a seven-membered ring (or triazepine derivative, i.e., an NH-NH arrangement). Apparently, once the ring opening takes place, the cyclization toward the six-membered ring is favored despite the fact that the a nitrogen, in the CO-N_aH-N_bH_2 moiety, should be a poorer nucleophile than the b nitrogen. Bearing in mind that the reaction does not require more than 1 equiv (mol per mol) of hydrazine, and no additives are needed to facilitate the closing step, it is also plausible that the b nitrogen of the intermediate is largely protonated and/or that transient species such as that depicted in Scheme 8 participate in the cyclization step.

Conclusions

N-Nitration of uridine and inosine derivatives can be accomplished in good yields, in cold CH_2Cl_2, with the nitrating agent generated from NH_4NO_3 and $(CF_3CO)_2O$, but not with other attempted nitrating agents. Since free hydroxyl groups are readily converted into nitrate esters, it is in principle recommendable to subject these nucleosides to nitration after protection as esters and/or acetals, which are stable under the reaction conditions.

In general, *N*-nitro inosine derivatives are much more reactive (as well as more sensitive to heating and light, see Experimental Section) than *N*-nitro uridine derivatives.

N-Nitro uridines and inosines react with nitrogen nucleophiles to afford open-ring intermediates, which in some cases have been fully characterized with the aid of ^{15}N-labeling experiments. Cyclization of these intermediates may lead again to the pyrimidine and purine systems, with replacement of NNO_2 by *NH, NR, NNH_2, or *N*NH_2.

The procedure is nothing else than an additional example of a well-known mechanistic topic, viz. a substitution reaction through a ring-opening/ring-closing process. However, all together this constitutes a mild, novel, and rather general method for the ^{15}N-labeling of relevant nitrogen atoms of pyrimidine and purine nucleosides as well as for the incorporation of a series of bulky and/or chiral chains on N1 of inosine, which seems amenable to further improvements and applications.

Experimental Section

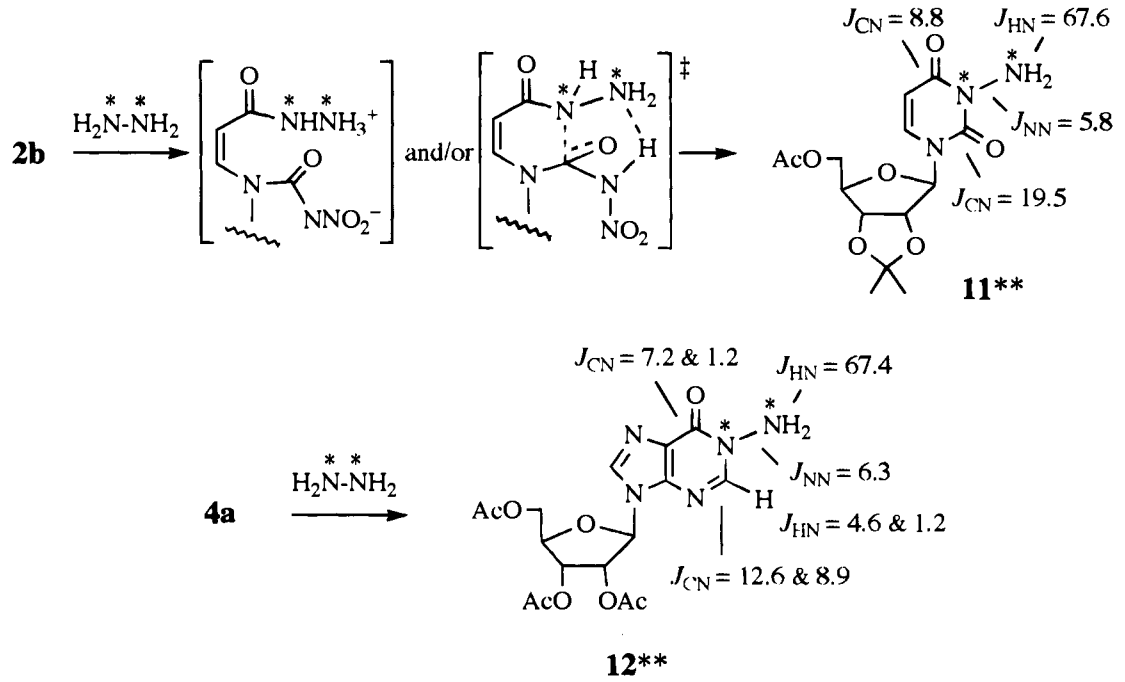
All reactions were carried out under Ar in oven- or flame-dried glassware, using standard syringe and septum equipment. Dichloromethane was distilled from CaH_2, methanol from magnesium (and stored over 3-Å molecular sieves), THF from Na/benzophenone, and acetonitrile from phosphorus(V) oxide (and stored over 3-Å molecular sieves). TLC plates (Merck silica gel 60 F_{254}) and HPLC analytical columns (Spherisorb W, silica gel) on a Shimadzu instrument were employed everywhere. Separations were carried out by "flash" column chromatography (SDS silica gel, 230−400 mesh) unless otherwise indicated. ^{1}H, ^{13}C-{^{1}H}, and DEPT NMR spectra were recorded on a Varian Gemini-200 instrument; chemical shifts are given in ppm relative to TMS and coupling constants in Hz. 2D NMR (^{13}C-^{1}H COSY, HMBC) experiments were performed on a Varian VXR-500 spectrometer. ^{15}N NMR spectra were registered on a Varian Unity-300 instrument; chemical shifts are referred to external concentrated

(18) The assignment of ^{13}C signals of C2 and C4 was previously confirmed by 2D NMR experiments. For long-range ^{1}H−^{13}C coupling constants in the nucleoside field, see: Uzawa, J.; Uramoto, M. *Org. Magn. Res.* **1979**, *12*, 612.

(19) Maeda, M.; Kawazoe, Y. *Chem. Pharm. Bull.* **1975**, *23*, 844. For related N-amination reactions, see: (b) Kuroda, T.; Hisamura, K.; Matsukuma, I.; Nishikawa, H.; Morimoto, M.; Ashizawa, T.; Nakamizo, N.; Otsuji, Y. *J. Heterocycl. Chem.* **1992**, *29*, 1133. (c) Maillard, M.; Florent, J. C.; Lemaître, M.; Begassat, F.; Bugnicourt, A.; Ferrieux, C.; Rombi, C.; Pacaud, E.; Thierry, D.; Zerial, A.; Monneret, C.; Grierson, D. S. *Biorg. Med. Chem. Lett.* **1992**, *2*, 1469.

(20) (a) Broom, A. D.; Robins, R. K. *J. Org. Chem.* **1969**, *34*, 1025. (b) For the conversion of adenosine into 1-aminoadenosine, see: Huang, G. F.; Okamoto, T.; Maeda, M.; Kawazoe, Y. *Tetrahedron Lett.* **1973**, 4541.

Scheme 8



$H^{15}NO_3$ (negative values upfield). Melting points (uncorrected) were obtained on a Büchi apparatus. Optical rotations were measured at 23–25 °C in $CHCl_3$ on a Perkin-Elmer 241 MC polarimeter. Mass spectra (CI or FAB) were obtained on a Hewlett-Packard 5988A spectrometer. Elemental analyses were performed either by the Serveis Científico-Tècnics (Universitat de Barcelona) or by the Servei de Microanàlisi del CID (Barcelona).

Uridine (either from Sigma or Aldrich), inosine (Sigma or Aldrich), adenosine (Scharlau), and guanosine (Scharlau) were protected according to standard procedures.[3a,b] $^{15}NH_4Cl$ was purchased either from IC Chemikalien (MSD Isotopes, 99% of label) or Aldrich (98% of label), and [$^{15}N_2$]hydrazinium sulfate from IC Chemikalien (99.6% of label).

N-Nitration. General procedure. Trifluoroacetic anhydride (0.560 mL, 4.0 mmol) was added to a suspension of finely powdered NH_4NO_3 (160 mg, 2.0 mmol) in anhydrous CH_2Cl_2 (5 mL), at 0 °C. The mixture was vigorously stirred at room temperature until the solid was dissolved (ca. 1 h) and then cooled again. Addition of the nucleoside (1.0 mmol in the case of **1a** and **1b**, 0.5 mmol in the remaining cases) and stirring at 0 °C (in the uridine series) or at −20 °C (in the inosine series) for 20–60 min, depending on the case (monitored by TLC), addition of more solvent, washing with cold phosphate buffer, drying, evaporation in vacuo, and filtration through a pad of silica gel (99:1 or 98:2 CH_2Cl_2–MeOH) afforded the pure *N*-nitro derivative (often as a white gum of low melting point, which did not crystallize).

2′,3′,5′-Tri-*O*-acetyl-3-nitrouridine (2a): $[\alpha]_D$ 18.0° (*c* 1.57); ^1H NMR ($CDCl_3$) δ 2.12, 2.13, 2.14 (3 s, 3 Me), 4.30–4.45 (m, H4′, H5′, H5″), 5.33 (m, H3′), 5.39 (m, H2′), 5.92 (d, J_{56} = 8.4, H5), 5.96 (d, $J_{1'2'}$ = 4.6, H1′), 7.52 (d, H6); ^{13}C NMR ($CDCl_3$) δ 20.2, 20.3, 20.6 (3 Me), 62.5 (C5′), 69.6 (C3′), 72.7 (C2′), 80.2 (C4′), 88.9 (C1′), 101.8 (C5), 139.3 (C6), 145.3 (C2), 154.8 (C4), 169.5, 169.6, 170.0 (3 COMe). Anal. Calcd for $C_{15}H_{17}N_3O_{11}$: C, 43.38; H, 4.13; N, 10.12. Found: C, 43.60; H, 4.28; N, 10.01.

5′-*O*-Acetyl-2′,3′-*O*-isopropylidene-3-nitrouridine (2b): $[\alpha]_D$ 17.6° (*c* 1.00); ^1H NMR ($CDCl_3$) δ 1.37, 1.58 (2 s, Me_2C), 2.08 (s, MeCO), 4.25 (dd, $J_{5'5''}$ = 12.0, $J_{4'5'}$ = 5.5, H5′), 4.30 (dd, $J_{4'5''}$ = 4.0, H5″), 4.37 (ddd, $J_{3'4'}$ = 3.8, H4′), 4.80 (dd, $J_{2'3'}$ = 6.4, H3′), 5.00 (dd, $J_{1'2'}$ = 2.2, H2′), 5.71 (d, H1′), 5.87 (d, J_{56} = 8.3, H5), 7.40 (d, H6); ^{13}C NMR ($CDCl_3$) δ 20.6 (MeCO), 25.1, 26.9 (Me_2C), 63.5 (C5′), 80.6 (C3′), 84.4 (C2′), 85.3 (C4′), 95.2 (C1′), 101.1 (C5), 114.8 (Me_2C), 141.2 (C6), 145.1 (C2), 155.0 (C4), 170.2 (COMe); assignments confirmed by 2D NMR experiments; CIMS *m/z* 389 ($M + NH_4^+$). Anal. Calcd for $C_{14}H_{17}N_3O_9$: C, 45.29; H, 4.61; N, 11.32. Found: C, 45.40; H, 4.61; N, 10.97.

[3-^{15}N]-5′-*O*-Acetyl-2′,3′-*O*-isopropylidene-3-nitrouridine (2b*). Prepared by nitration of **1b*** according to the general procedure described above: ^1H NMR ($CDCl_3$) δ 1.37, 1.57 (2 s, Me_2C), 2.09 (s, MeCO), 4.2–4.5 (m, H4′, H5′, H5″), 4.81 (dd, $J_{2'3'}$ = 6.4, $J_{3'4'}$ = 3.8, H3′), 5.01 (dd, $J_{1'2'}$ = 2.1, H2′), 5.69 (d, H1′), 5.87 (dd, J_{56} = 8.3, $J_{N,5}$ = 4.1, H5), 7.36 (d, H6); ^{13}C NMR ($CDCl_3$) δ 20.7 (MeCO), 25.3, 27.0 (Me_2C), 63.6 (C5′), 80.7 (C3′), 84.5 (C2′), 85.5 (C4′), 95.6 (C1′), 101.3 (d, J_{CN} = 8.9, C5), 114.9 (Me_2C), 141.2 (C6), 145.1 (d, J_{CN} = 13.6, C2), 155.0 (d, J_{CN} = 2.7, C4), 170.2 (COMe).

2′,3′-*O*-Isopropylidene-N^3,O^5′-dinitrouridine (2c): $[\alpha]_D$ 38.4° (*c* 1.00); ^1H NMR ($CDCl_3$) δ 1.37, 1.57 (2 s, Me_2C), 4.42 (m, H4′), 4.63–4.75 (m, H5′, H5″), 4.88 (dd, $J_{2'3'}$ = 6.4, $J_{3'4'}$ = 4.1, H3′), 5.08 (dd, $J_{1'2'}$ = 1.8, H2′), 5.65 (d, H1′), 5.90 (d, J_{56} = 8.3, H5), 7.31 (d, H6); ^{13}C NMR ($CDCl_3$) δ 25.0, 26.9 (Me_2C), 71.8 (C5′), 80.8 (C3′), 84.0 (C2′ or C4′), 84.5 (C4′ or C2′), 96.4 (C1′), 101.7 (C5), 115.2 (Me_2C), 142.1 (C6), 145.3 (C2), 154.9 (C4). Anal. Calcd for $C_{12}H_{14}N_4O_{10}$: C, 38.51; H, 3.77; N, 14.97. Found: C, 38.76; H, 3.90; N, 14.71.

2′,3′,5′-Tri-*O*-benzoyl-3-nitrouridine (2d): mp 162–163 °C (iPrOH); $[\alpha]_D$ −45.7° (*c* 1.00); ^1H NMR ($CDCl_3$) δ 4.64–4.88 (m, H4′, H5′, H5″), 5.73 (d, J_{56} = 8.3, H5), 5.79 (m, H2′), 5.92 (m, H3′), 6.23 (d, $J_{1'2'}$ = 5.2, H1′), 7.2–8.2 (m, H6, 3 Ph); ^{13}C NMR ($CDCl_3$) δ 63.2 (C5′), 70.7 (C3′), 73.8 (C2′), 80.7 (C4′), 89.7 (C1′), 102.0 (C5), 128.3–129.8 (3 C_o, 3 C_m, 3 C_i), 133.7, 133.8, 133.9 (C_p), 139.5 (C6), 145.3 (C2), 154.8 (C4), 165.2, 165.3, 165.9 (3 COPh). Anal. Calcd for $C_{30}H_{23}N_3O_{11}$: C, 59.90; H, 3.85; N, 6.99. Found: C, 60.04; H, 3.90; N, 6.76.

3-Nitrouridine (2). Treatment of either **2a** or **2b** (1 mmol) with a saturated solution of HCl (g) in MeOH (10 mL) overnight at room temperature, followed by evaporation of the solvent in vacuo, co-evaporation with toluene (at 0.1 mm Hg), and purification by column chromatography (9:1 CH_2Cl_2–MeOH) afforded **2** in 75–80% yields: mp 161 °C (dec) (AcOEt); ^1H NMR (CD_3OD) δ 3.81 (dd, $J_{5'5''}$ = 12.2, $J_{4'5'}$ = 3.0, H5′), 3.92 (dd, $J_{4'5''}$ = 2.5, H5″), 4.11 (m, H4′), 4.12–4.29 (m, H2′, H3′), 5.94 (d, $J_{1'2'}$ = 4.4, H1′), 5.96 (d, J_{56} = 8.4, H5), 8.22 (d, H6); ^{13}C NMR (CD_3OD): δ 61.8 (C5′), 71.0 (C2′), 75.8 (C3′), 86.7 (C4′), 91.6 (C1′), 101.5 (C5), 142.3 (C6), 147.1 (C2), 157.1 (C4). Anal. Calcd for $C_9H_{11}N_3O_8$: C, 37.38; H, 3.83; N, 14.53. Found: C, 37.49; H, 3.90; N, 14.30.

2′,3′,5′-Tri-*O*-acetyl-1-nitroinosine (4a): colorless crystals that turn yellow on exposure to the light; dec. 155 °C (MeOH); $[\alpha]_D$ −26.6° (*c* 0.89); ^1H NMR ($CDCl_3$) δ 2.11, 2.14, 2.16 (3 s, 3 Me), 4.30–4.50 (m, H4′, H5′, H5″), 5.54 (dd, $J_{2'3'}$ = 5.5, $J_{3'4'}$ = 4.9, H3′), 5.80 (dd, $J_{1'2'}$ = 5.1, H2′), 6.14 (d, H1′), 8.07 (s, H8), 8.72 (s, H2); ^{13}C NMR ($CDCl_3$) δ 20.3, 20.4, 20.7 (3 Me), 62.8 (C5′), 70.2 (C3′), 73.4 (C2′), 80.4 (C4′),

86.8 (C1′), 125.3 (C5), 139.8 (C8), 141.2 (C2), 145.1 (C6), 149.4 (C4), 169.3, 169.5, 170.2 (3 COMe); assignments confirmed by 2D NMR experiments; FABMS *m/z* 440 (M + 1$^+$). Anal. Calcd for $C_{16}H_{17}N_5O_{10}$: C, 43.74; H, 3.90; N, 15.94. Found: C, 43.81; H, 3.99; N, 15.95.

5′-*O*-Acetyl-2′,3′-*O*-isopropylidene-1-nitroinosine (4b): white gum that turns yellow on exposure to the light; $[\alpha]_D$ −26.7° (*c* 0.81); ^{1}H NMR ($CDCl_3$) δ 1.40, 1.63 (2 s, Me_2C), 2.05 (s, MeCO), 4.26 (dd, $J_{4'5'}$ = 5.5, $J_{5'5''}$ = 12.1, H5′), 4.35 (dd, $J_{4'5''}$ = 4.0, H5″), 4.54 (ddd, $J_{3'4'}$ = 3.4, H4′), 4.94 (dd, $J_{2'3'}$ = 6.3, H3′), 5.20 (dd, $J_{1'2'}$ = 2.5, H2′), 6.14 (d, H1′), 8.03 (s, H8), 8.72 (s, H2); ^{13}C NMR ($CDCl_3$) δ 20.6 (MeCO), 25.2, 27.1 (Me_2C), 63.8 (C5′), 81.1 (C3′), 84.7 (C2′), 84.9 (C4′), 91.1 (C1′), 115.1 (Me_2C), 125.2 (C5), 140.0 (C8), 141.1 (C2), 144.7 (C6), 149.4 (C4), 170.3 (COMe). Anal. Calcd for $C_{15}H_{17}N_5O_8$: C, 45.57; H, 4.33; N, 17.72. Found: C, 45.82; H, 4.50; N, 17.49.

[3-^{15}N]-5′-*O*-Acetyl-2′,3′-*O*-isopropylideneuridine (1b*). To a stirred solution of $^{15}NH_4Cl$ (65 mg, 1.2 mmol) and KOH (85%, 66 mg, 1.0 mmol) in water (2.5 mL) was added Et_3N (167 μL, 1.2 mmol) and acetonitrile (2.5 mL). A solution of nitrouridine **2b** (371 mg, 1.0 mmol) in acetonitrile (5 mL) was then introduced. After stirring for 5 days at room temperature the solvent was removed in vacuo and the residue was separated by column chromatography with 95:5 CH_2Cl_2−MeOH to give **1b*** (240 mg, 73%):[15a] R_f and mp identical to those of the unlabeled compound (**1b**); ^{1}H NMR ($CDCl_3$) δ 1.37, 1.58 (2 s, Me_2C), 2.10 (s, MeCO); 4.2−4.4 (m, H4′, H5′, H5″), 4.81 (dd, $J_{2'3'}$ = 6.4, $J_{3'4'}$ = 3.7, H3′), 5.00 (dd, $J_{1'2'}$ = 1.9, H2′), 5.64 (d, H1′), 5.74 (ddd, J_{56} = 8.1, $J_{5,N}$ = 2.8, J_{35} = 2.1, H5), 7.27 (d, H6), 8.51 (dd, J_{HN} = 91.1, NH); ^{13}C NMR ($CDCl_3$) δ 20.7 (MeCO), 25.1, 27.0 (Me_2C), 64.2 (C5′), 81.1 (C3′), 84.5 (C2′), 85.4 (C4′), 95.1 (C1′), 102.4 (d, J_{CN} = 6.7, C5), 114.4 (Me_2C), 142.4 (C6), 150.0 (d, J_{CN} = 18.0, C2), 163.7 (d, J_{CN} = 9.6, C4), 170.4 (MeCO); ^{15}N NMR ($CDCl_3$) δ −210.6 (dd, J_{NH} = 91.0, $J_{N,5}$ = 2.7).

[3-^{15}N]Uridine (1*). Compound **1b*** (44 mg, 0.13 mmol) was dissolved in a saturated solution of HCl (g) in MeOH (1 mL). After stirring for 24 h, the solvent was evaporated in vacuo to give a chromatographically pure solid, **1*** (32 mg, 97%): R_f and mp identical to those of uridine (**1**); ^{1}H NMR (CD_3SOCD_3) δ 3.48−3.64 (m, H5′, H5″), 3.82 (m, H4′), 3.94−4.02 (m, H2′, H3′), 5.63 (ddd, J_{56} = 8.1, $J_{5,N}$ = 2.5, J_{35} = 2.2, H5), 5.76 (d, $J_{1'2'}$ = 5.2, H1′), 7.88 (d, H6), 11.31 (dd, J_{HN} = 89.8, NH); ^{13}C NMR (CD_3SOCD_3) δ 60.9 (C5′), 69.9 (C2′), 73.6 (C3′), 84.9 (C4′), 87.7 (C1′), 101.8 (d, J_{CN} = 6.3, C5), 140.8 (C6), 150.8 (d, J_{CN} = 17.4, C2), 163.2 (d, J_{CN} = 9.1, C4); ^{15}N NMR (CD_3SOCD_3) δ −212.6 (d, J_{NH} = 90.9).

[1-^{15}N]-2′,3′,5′-Tri-*O*-acetylinosine (3a*). To a solution of $^{15}NH_4Cl$ (12 mg, 0.22 mmol) and KOH (85%, 13 mg, 0.20 mmol) in water (1 mL) was added Et_3N (27 μL, 0.20 mmol). A solution of nitroinosine **4a** (84 mg, 0.19 mmol) in acetonitrile (3 mL) was then poured into it. After stirring for 6 h at room temperature the solvent was evaporated in vacuo and the residue was purified by column chromatography with 95:5 CH_2Cl_2−MeOH to give **3a*** (63 mg, 84%): R_f and mp identical to those of triacetylated inosine (**3a**); ^{1}H NMR ($CDCl_3$) δ 2.10, 2.15, 2.16 (3 s, 3 Me), 4.30−4.50 (m, H4′, H5′, H5′), 5.61 (dd, $J_{2'3'}$ = 5.5, $J_{3'4'}$ = 4.5, H3′), 5.88 (dd, $J_{1'2'}$ = 5.2, H2′), 6.17 (d, H1′), 8.01 (s, H8), 8.24 (d, $J_{2,N}$ = 7.6, H2), 13.06 (d, J_{HN} = 87.5, NH); ^{13}C NMR ($CDCl_3$) δ 20.3, 20.4, 20.7 (3 Me), 62.9 (C5′), 70.4 (C3′), 73.2 (C2′), 80.3 (C4′), 86.4 (C1′), 125.2 (C5), 138.5 (C8), 145.9 (d, J_{CN} = 8.2, C2), 148.7 (C4), 158.8 (d, J_{CN} = 10.6, C6), 169.2, 169.5, 170.3 (3 COMe); ^{15}N NMR ($CDCl_3$) δ −198.8.

[1-^{15}N]Inosine (3*). To a solution of **3a*** (198 mg, 0.5 mmol) in MeOH (5 mL) was added a saturated solution of HCl (g) in MeOH (5 mL). Stirring was maintained overnight at room temperature. After evaporation of the solvent in vacuo, the residue was taken up in 1:1 MeOH−H_2O (10 mL) and treated with a weakly basic ion-exchange resin (Amberlyst A-21) until neutralization. Filtration and removal of the solvent under vacuum gave **3*** (131 mg, 97%): R_f and mp identical to those of inosine (**3**); ^{1}H NMR (CD_3SOCD_3) δ 3.50−3.70 (m, H5′, H5′), 3.92 (m, H4′), 4.11 (m, H3′), 4.47 (m, H2′), 5.06 (t, $J_{OH,5'}$ = $J_{OH,5''}$ = 5.5, 5′-OH), 5.19 (d, *J* = 4.9, OH), 5.48 (d, *J* = 6.1, OH), 5.86 (d, $J_{1'2'}$ = 5.8, H1′), 8.06 (d, $J_{2,N}$ = 7.1, H2), 8.33 (s, H8), 12.38 (d, J_{HN} = 89.7, NH); ^{13}C NMR (CD_3SOCD_3) δ 61.2 (C5′), 70.3 (C2′), 74.1 (C3′), 85.6 (C4′), 87.4 (C1′), 124.4 (d, J_{CN} = 7.8, C5), 138.7 (C8), 145.8 (d, J_{CN} = 8.4, C2), 148.2 (d, J_{CN} = 2.0, C4), 156.5 (d, J_{CN} = 9.7, C6); ^{15}N NMR (CD_3SOCD_3) δ −192.0.

[1-^{15}N]Adenosine (5*). A mixture of anhydrous DMF (130 μL) and $SOCl_2$ (260 μL) in CH_2Cl_2 (4 mL) was added to a solution of **4a*** (130 mg, 0.33 mmol) in refluxing CH_2Cl_2, under N_2. After 3 h, the resulting solution was poured into an aqueous suspension of $NaHCO_3$ (0.5 g). Extraction with CH_2Cl_2, drying of the organic extracts over Na_2SO_4, evaporation of the solvent, and filtration through a pad of silica gel with 99:1 CH_2Cl_2−MeOH gave [1-^{15}N]-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (137 mg, 100%): ^{1}H NMR ($CDCl_3$) δ 2.10, 2.13, 2.17 (3 s, 3 Me), 4.30−4.55 (m, H4′, H5′, H5″), 5.66 (dd, $J_{2'3'}$ = 5.6, $J_{3'4'}$ = 4.5, H3′), 5.96 (dd, $J_{1'2'}$ = 5.1, H2′), 6.25 (d, H1′), 8.34 (s, H8), 8.79 (d, $J_{2,N}$ = 15.8, H2); ^{13}C NMR ($CDCl_3$) δ 20.3, 20.4, 20.6 (3 Me), 62.8 (C5′), 70.4 (C3′), 73.0 (C2′), 80.4 (C4′), 86.8 (C1′), 132.2 (d, J_{CN} = 2.6, C5), 143.6 (C8), 151.1 (d, J_{CN} = 2.6, C4), 151.4 (d, J_{CN} = 4.7, C6), 152.2 (d, J_{CN} = 4.0, C2), 169.2, 169.5, 170.2 (3 COMe); ^{15}N NMR ($CDCl_3$) δ −96.1.

A sample of this chloro derivative (97 mg, 0.23 mmol) was treated with a saturated methanolic solution of ammonia (4 mL) in a sealed tube, for 5 h at 120 °C. Silica gel was added, and the solvent was removed in vacuo. Purification of the residue by column chromatography (9:1 to 8:2 CH_2Cl_2−MeOH) yielded N1-labeled adenosine, **5*** (55 mg, 87%): R_f and mp identical to those of adenosine; ^{1}H NMR (CD_3SOCD_3) δ 3.50−3.70 (m, H5′, H5″), 3.95 (m, H4′), 4.13 (m, H3′), 4.60 (m, H2′), 5.18 (d, *J* = 4.4, OH), 5.43−5.46 (m, 2 OH), 5.86 (d, $J_{1'2'}$ = 6.1, H1′), 7.34 (br s, NH_2), 8.12 (d, $J_{2,N}$ = 15.7, H2), 8.34 (s, H8); ^{13}C NMR (CD_3SOCD_3) δ 61.6 (C5′), 70.6 (C2′), 73.4 (C3′), 85.8 (C4′), 87.9 (C1′), 119.3 (C5), 139.9 (C8), 149.0 (C4), 152.3 (C2), 156.1 (d, J_{CN} = 4.0, C6); ^{15}N NMR (CD_3SOCD_3) δ −130.8.

Reactions of 2b with Benzylamine and with ^{15}N-Labeled Benzylamine. Benzylamine (23 μL, 0.21 mmol) and anhydrous K_2CO_3 (28 mg, 0.20 mmol) were added to a solution of **2b** (74 mg, 0.20 mmol) in acetonitrile (5 mL) at room temperature. After 20 h, the mixture was heated at 80 °C for 3 h. The solution was poured into a phosphate buffer solution (pH 7), was extracted with CH_2Cl_2, the organic extracts were dried over Na_2SO_4, and evaporated, and the residue was purified by column chromatography with 98:2 CH_2Cl_2−MeOH to afford 5′-*O*-acetyl-3-benzyl-2′,3′-*O*-isopropylideneuridine, **7** (59 mg, 71%): $[\alpha]_D$ 7.6° (*c* 2.29); ^{1}H NMR ($CDCl_3$) δ 1.36, 1.57 (2 s, Me_2C), 2.04 (s, MeCO), 4.2−4.4 (m, H4′, H5′, H5″), 4.81 (dd, $J_{2'3'}$ = 6.5, $J_{3'4'}$ = 3.7, H3′), 4.96 (dd, $J_{1'2'}$ = 1.8, H2′), 5.05 (d, *J* = 13.7, NC*H*HPh), 5.13 (d, NCH*H*Ph), 5.64 (d, H1′), 5.77 (d, J_{56} = 8.0, H5), 7.20−7.35 (m, 3 H arom, H6), 7.4−7.5 (m, 2 H arom); ^{13}C NMR ($CDCl_3$) δ 20.7 (MeCO), 25.3, 27.1 (Me_2C), 44.0 (N-CH_2), 64.2 (C5′), 81.1 (C3′), 84.8 (C2′), 85.4 (C4′), 95.8 (C1′), 102.0 (C5), 114.4 (Me_2C), 127.6, 128.4, 129.0 (CH arom), 136.3 (C_i), 139.7 (C6), 150.6 (C2), 162.4 (C4), 170.3 (COMe). Anal. Calcd for $C_{21}H_{24}N_2O_7$: C, 60.57; H, 5.81; N, 6.73. Found: C, 60.48; H, 6.01; N, 6.61.

To a solution of **2b** (50 mg, 0.134 mmol) in $CDCl_3$ (0.7 mL) was added benzylamine (30 μL, 0.273 mmol). After 24 h the major product (**6**) showed the following spectroscopical data: ^{1}H NMR δ 1.26, 1.46 (2 s, Me_2C), 1.90 (s, MeCO), 4.00−4.06 (m, H4′, H5′, NH_3CH_2), 4.16−4.28 (m, H5″, $CONHCH_2$), 4.60 (dd, $J_{3'4'}$ = 4.5, $J_{2'3'}$ = 6.5, H3′), 4.87 (dd, $J_{1'2'}$ = 2.5, H2′), 5.49 (d, J_{56} = 9.0, H5), 5.64 (d, H1′), 6.25 (d, H6), 6.39 (br s, NH_3), 7.15−7.37 (m, 10 H arom, CONH); ^{13}C NMR δ 20.7 (MeCO), 25.4, 27.2 (Me_2C), 43.2 ($CONHCH_2$), 43.8 (NH_3CH_2), 64.6 (C5′), 81.0 (C3′), 83.2 (C4′), 83.3 (C2′), 93.8 (C1′), 113.9 (Me_2C), 116.8 (C5), 127.5−128.9 (CH arom), 133.6 (C6), 134.8, 137.9 (2 C_i), 159.4 (C2), 165.4 (C4), 170.9 (COMe); assignments corroborated by ^{13}C−^{1}H 2D NMR.

For parallel experiments with labeled samples, see Scheme 6. Reaction of **2b** with ^{15}N-labeled benzylamine was monitored by ^{13}C NMR; the following splittings were noted (compound **6****): 43.2 (J_{CN} = 11.0), 43.8 (J_{CN} = 5.0), 116.7 (J_{CN} = 7.7), 165.4 (J_{CN} =16.4).

Reactions of 4a with Benzylamine and with ^{15}N-Labeled Benzylamine. To a solution of nitro compound **4a** (132 mg, 0.30 mmol) in CH_2Cl_2 (6 mL) at −78 °C was added benzylamine (72 μL, 0.66 mmol). After 90 min, CF_3COOH (52 μL, 0.68 mmol) was added, and the mixture was allowed to warm up to room temperature. Seven hours later, the solvent was removed in vacuo, and the residue was separated by column chromatography with 98:2 CH_2Cl_2−MeOH to give 2′,3′,5′-tri-*O*-acetyl-1-benzylinosine (**8**, R = CH_2Ph) (132 mg, 91%):

$[\alpha]_D$ −29.0° (*c* 3.34); ^1H NMR ($CDCl_3$) δ 2.08, 2.09, 2.13 (3 s, 3 Me), 4.28−4.46 (m, H4′, H5′, H5″), 5.26 (s, N-CH_2), 5.61 (dd, $J_{2'3'}$ = 5.5, $J_{3'4'}$ = 4.8, H3′), 5.89 (dd, $J_{1'2'}$ = 5.0, H2′), 6.07 (d, H1′), 7.30−7.40 (m, Ph), 7.96 (s, H8), 8.11 (s, H2); ^{13}C NMR ($CDCl_3$) δ 20.2, 20.3, 20.5 (3 Me), 49.0 (N-CH_2), 62.8 (C5′), 70.1 (C3′), 73.0 (C2′), 79.9 (C4′), 86.4 (C1′), 125.2 (C5), 128.0, 128.1, 128.8 (CH arom), 135.6 (C_i), 138.5 (C8), 146.8 (C4), 147.3 (C2), 156.2 (C6), 169.1, 169.4, 170.1 (3 COMe). Anal. Calcd for $C_{23}H_{24}N_4O_8$: C, 57.02; H, 4.99; N, 11.56. Found: C, 57.25; H, 5.11; N, 11.38.

To a refluxing solution of benzylamine (130 μL, 1.2 mmol) in $CHCl_3$ (10 mL) was added **4a** (176 mg, 0.40 mmol). A few minutes later, the solvent was removed, and the residue was separated by column chromatography as above to afford an open bis(benzylamino) derivative, 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-*N*-benzyl-5-[(*N*-benzylamino)-methylene]aminoimidazole-4-carboxamide, **10** (103 mg, 53%): ^1H NMR ($CDCl_3$) δ 1.97, 2.06, 2.12 (3 s, 3 MeCO), 4.22−4.36 (m, H4′, H5′, H5″), 4.49−4.69 (m, CONHCH_2, CHNHCH_2), 5.36 (dd, $J_{2'3'}$ = 5.4, $J_{3'4'}$ = 5.8, H3′), 5.66 (dd, $J_{1'2'}$ = 4.2, H2′), 5.95 (d, H1′), 6.40 (m, N*H*-CH), 7.26−7.35 (m, 2 Ph), 7.41 (s, H2), 7.51 (t, CONH), 9.02 (d, *J* = 4.1, N=C*H*-NH); ^{13}C NMR ($CDCl_3$) δ 20.2, 20.4, 20.7 (3 Me), 42.6, 44.1 (2 N-CH_2), 62.6 (C5′), 69.6 (C3′), 73.6 (C2′), 78.8 (C4′), 86.0 (C1′), 119.0 (C4), 127.0, 127.1, 127.4, 127.5, 128.4, 128.4, 128.8 (CH arom, C2), 138.1, 138.6 (2 Ci), 144.7 (C5), 156.4 (N=CH-NH), 163.8 (CONH), 169.1, 169.4, 170.3 (3 COMe).

^{15}N-Labeled benzylamine (22 μL, 0.20 mmol) was introduced into a solution of **4a** (43 mg, 0.10 mmol) in $CDCl_3$ (0.7 mL) at −40 °C, and the changes were monitored by ^1H and ^{13}C NMR. A polar intermediate (**9****) was formed. When the reaction was allowed to warm up to room temperature, **9**** slowly disappeared (in this experiment CF_3COOH was not utilized) and it was converted into **8***, R = CH_2Ph. NMR data of **9****: ^1H NMR ($CDCl_3$) δ 1.90, 1.98, 2.03 (3 s, 3 MeCO), 4.03−4.62 (m, NH_3CH_2, H4′, H5′, H5″, CHNHCH_2), 5.28 (dd, $J_{2'3'}$ = 5.7, $J_{3'4'}$ = 5.1, H3′), 5.55 (dd, $J_{1'2'}$ = 4.2, H2′), 5.79 (d, H1′), 7.10−7.27 (m, H arom, H2, N*H*CH_2), 8.49 (dd, $J_{2,N}$ = 7.5, $J_{2,NH}$ = 3.6, N=C*H*-NH); ^{13}C NMR ($CDCl_3$) δ 20.2, 20.4, 20.6 (3 Me), 43.5 (d, J_{CN} = 2.1, NH_3CH_2), 44.0 (d, J_{CN} = 9.8, $NHCH_2$), 62.9 (C5′), 69.8 (C3′), 73.5 (C2′), 78.8 (C4′), 85.9 (C1′), 120.7 (C4), 126.9−128.9 (CH arom), 134.1, 138.5 (2 C_i), 147.2 (C5), 157.1 (d, J_{CN} = 18.3, N=CH-NH), 168.4 (C6), 169.2, 169.4, 170.5 (3 COMe). In **10****, as compared to **10**, the following splittings were noted: δ 6.40 (J_{HN} = 94.0), 9.02 (J_{HN} = 8.3), 42.6 (J_{CN} = 11.9), 44.1 (J_{CN} = 10.2), 156.4 (J_{CN} = 18.0).

Reaction of 4a with Methyl Glycinate. Nitroinosine **4a** (132 mg, 0.30 mmol) in CH_2Cl_2 (6 mL) and methyl glycinate (59 mg, 0.66 mmol) in CH_2Cl_2 (1 mL) were mixed at −78 °C and then stirred overnight at −20 °C. After addition of CF_3COOH (52 μL, 0.68 mmol), the mixture was stirred at room temperature for 10 h. Removal of the solvent in vacuo and purification of the residue by column chromatography gave **8**, R = CH_2COOMe (134 mg, 96%): $[\alpha]_D$ −34.2° (*c* 0.56); ^1H NMR ($CDCl_3$) δ 2.11, 2.13, 2.15 (3 s, 3 MeCO), 3.79 (s, OMe), 4.30−4.45 (m, H4′, H5′, H5″), 4.83 (s, N-CH_2), 5.64 (dd, $J_{2'3'}$ = 5.5, $J_{3'4'}$ = 4.7, H3′), 5.90 (dd, $J_{1'2'}$ = 5.2, H2′), 6.13 (d, H1′), 7.97 (s, H8), 8.04 (s, H2); ^{13}C NMR ($CDCl_3$) δ 20.3, 20.4, 20.6 (3 MeCO), 46.9 (N-CH_2), 52.8 (OMe), 62.9 (C5′), 70.3 (C3′), 73.1 (C2′), 80.1 (C4′), 86.4 (C1′), 125.0 (C5), 138.6 (C8), 147.2 (C4), 147.7 (C2), 156.0 (C6), 167.7 (COOMe), 169.2, 169.5, 170.3 (3 COMe). Anal. Calcd for $C_{19}H_{22}N_4O_{10}$: C, 48.92; H, 4.76; N, 12.02. Found: C, 49.23; H, 4.98; N, 11.97.

Reaction of 4a with Methyl Alaninate. Nitroinosine **4a** (132 mg, 0.30 mmol) in CH_2Cl_2 (6 mL) and methyl L-alaninate (62 mg, 0.60 mmol) in CH_2Cl_2 (1 mL) were mixed at −78 °C and left overnight at −20 °C. The mixture was then allowed to warm to room temperature and was stirred for 2 days (until no **4a** remained). Treatment with CF_3COOH (46 μL, 0.60 mmol) for 35 h, removal of the solvent, and separation by column chromatography afforded **8**, R = (*S*)-CHMe-COOMe (115 mg, 80%): $[\alpha]_D$ −32.5° (*c* 2.46); ^1H NMR ($CDCl_3$) δ 1.76 (d, $J_{Me,H}$ = 7.4, CH_3CH), 2.12, 2.12, 2.15 (3 s, 3 MeCO), 3.78 (s, OMe), 4.30−4.48 (m, H4′, H5′, H5″), 5.63 (q, N-CH), 5.64 (dd, $J_{2'3'}$ = 5.5, $J_{3'4'}$ = 4.7, H3′), 5.90 (dd, $J_{1'2'}$ = 5.2, H2′), 6.13 (d, H1′), 7.98 (s, H8), 8.10 (s, H2); ^{13}C NMR ($CDCl_3$) δ 17.5 (CH_3CH), 20.4, 20.5, 20.7 (3 MeCO), 52.0 (N-CH), 53.0 (OMe), 62.9 (C5′), 70.2 (C3′), 73.2 (C2′), 80.1 (C4′), 86.7 (C1′), 124.9 (C5), 138.6 (C8), 146.0 (C2), 146.8 (C4), 155.1 (C6), 169.3, 169.5, 170.3 (3 COMe), 170.5 (COOMe). Anal. Calcd for $C_{20}H_{24}N_4O_{10}$: C, 50.00; H, 5.04; N, 11.66. Found: C, 50.19; H, 5.28; N, 11.36.

[3-^{15}N,$^{15}NH_2$]-5′-*O*-Acetyl-3-amino-2′,3′-*O*-isopropylideneuridine (11).** A solution of **2b** (76 mg, 0.20 mmol) in MeOH (5 mL) was added to a mixture of [$^{15}N_2$]hydrazinium sulfate (32 mg, 0.24 mmol), water (1 mL), and 1 M methanolic KOH (480 μL, 0.48 mmol) at room temperature. After stirring for 2 h, the resulting solution was partitioned between water and CH_2Cl_2. Organic extracts were dried, and the solvent was removed in vacuo to give chromatographically pure **11**** (60 mg, 85%): $[\alpha]_D$ 16.8° (*c* 2.02); ^1H NMR ($CDCl_3$) δ 1.36, 1.58 (2 s, Me_2C), 2.08 (s, MeCO), 4.2−4.5 (m, H4′, H5′, H5″), 4.85 (dd, $J_{2'3'}$ = 6.4, $J_{3'4'}$ = 3.8, H3′), 5.03 (dd, $J_{1'2'}$ = 1.8, H2′), 5.13 (d, J_{HN} = 67.6, NH_2), 5.64 (d, H1′), 5.85 (dd, J_{56} = 8.1, $J_{5,N}$ = 3.6, H5), 7.24 (d, H6); ^{13}C NMR ($CDCl_3$) δ 20.7 (MeCO), 25.1, 27.0 (*Me*$_2$C), 64.1 (C5′), 81.1 (C3′), 84.7 (C2′), 85.7 (C4′), 96.1 (C1′), 100.7 (d, J_{CN} = 7.7, C5), 114.4 (Me$_2$*C*), 138.7 (C6), 148.2 (d, J_{CN} = 19.5, C2), 159.5 (d, J_{CN} = 8.8, C4), 170.4 (COMe); ^{15}N NMR ($CDCl_3$) δ −188.3 (dd, J_{NN} = 5.8, $J_{N,5}$ = 3.6, N3), −302.9 (td, J_{NH} = 67.6, J_{NN} = 5.8, NH_2). A sample of the unlabeled compound (**11**) was submitted to microanalysis. Anal. Calcd for $C_{14}H_{19}N_3O_7$: C, 49.26; H, 5.61; N, 12.31. Found: C, 49.14; H, 5.77; N, 12.05.

[1-^{15}N,$^{15}NH_2$]-2′,3′,5′-Tri-*O*-acetyl-1-aminoinosine (12).** A solution of **4a** (88 mg, 0.20 mmol) in acetonitrile (3 mL) was added to a mixture of [$^{15}N_2$]hydrazinium sulfate (26 mg, 0.20 mmol), KOH (85%, 26 mg, 0.4 mmol), water (1 mL), and acetonitrile (1 mL) stirred at −15 °C. After 3 h the solvent was evaporated in vacuo, and the residue was purified by column chromatography (95:5 CH_2Cl_2−MeOH) to afford **12**** (62 mg, 76%): $[\alpha]_D$ −27.7° (*c* 0.94); ^1H NMR ($CDCl_3$) δ 2.10, 2.13, 2.15 (3 s, 3 Me), 4.30−4.50 (m, H4′, H5′, H5″), 5.01 (d, J_{HN} = 67.4, NH_2), 5.59 (dd, $J_{2'3'}$ = 5.5, $J_{3'4'}$ = 4.5, H3′), 5.87 (dd, $J_{1'2'}$ = 5.2, H2′), 6.10 (d, H1′), 7.96 (s, H8), 8.32 (dd, $J_{2,N1}$ = 4.6, $J_{2,NH2}$ = 1.2, H2); ^{13}C NMR ($CDCl_3$) δ 20.3, 20.5, 20.7 (3 Me), 63.0 (C5′), 70.5 (C3′), 73.2 (C2′), 80.3 (C4′), 86.5 (C1′), 124.8 (d, J_{CN} = 9.1, C5), 138.9 (C8), 147.2 (d, J_{CN} = 1.7, C4), 148.3 (dd, J_{CN} = 8.9, J_{CN} = 12.6, C2), 156.9 (dd, J_{CN} = 7.2, J_{CN} = 1.2, C6), 169.2, 169.5, 170.3 (3 COMe); ^{15}N NMR ($CDCl_3$) δ −181.9 (br d, J_{NN} = 6.3, N1), −304.8 (td, J_{NH} = 67.4, J_{NN} = 6.3, NH_2). A sample of the unlabeled compound, prepared from **4a** and hydrazinium sulfate, afforded the following microanalysis. Anal. Calcd for $C_{16}H_{19}N_5O_8$: C, 46.95; H, 4.68; N, 17.11. Found: C, 46.69; H, 4.90; N, 16.98.

Acknowledgment. Thanks are due to the Dirección General de Investigación Científica y Técnica (DGICYT) for financial support (Grants PB86-0137 and PB89-0277) and for FPI studentships to V.B. (1988−90) and X.A. (1990−93). We are also indebted to Dr. V. Tereshko and Dr. J. L. Campos, Universitat Politècnica, Barcelona, for the X-ray analysis of compound **4a**.

Supplementary Material Available: X-ray structure of 1-nitroinosine **4a**, with a selection of its bond lengths and angles and a summary of the main crystallographic data, reaction of **2a** with methylamine, **2b** with butylamine, **4a** with methylamine, butylamine, isopropylamine, *tert*-butylamine, methyl L-valinate, and methyl L-isoleucinate, and of **4b** with benzylamine, and preparation of ^{15}N-labeled benzylamine (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA943653M