24. Synthesis of \((^{15}\text{N}_2)^{17}\text{O}]\text{Urea, (}^{15}\text{N}_2[^{17}\text{O}]\text{Uridine, and (}^{15}\text{N}_2[^{17}\text{O}]\text{Cytidine)}\)

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A general synthetic approach for the synthesis of \(^{15}\text{N}-\) and \(^{17}\text{O}-\)doubly labelled pyrimidine nucleosides is described. The \(^{15}\text{N}\) isotopes in uridine and the \(^{17}\text{O}\) isotope in the urea-derived carbonyl group of uridine and cytidine originate from \((^{15}\text{N}_2[^{17}\text{O}]\text{urea (5) which was synthesized from}^{15}\text{NH}_2\text{Cl, thiophosgene (1), and}^{17}\text{H}_2\text{[}^{17}\text{O]}\). The third \(^{15}\text{N}\) isotope of cytidine in 4-position stems from the substitution of the 1,2,4-triazole moiety of \((^{15}\text{N}_2[^{17}\text{O}]\text{uridine derivative (8a/b}) with \(^{15}\text{NH}_2\text{OH. Hydrolysis of the same key intermediate (8a/b) with Na[}^{17}\text{OH/}^{17}\text{H}_2\text{]}\) introduced the second \(^{17}\text{O}\) isotope into the 4-position of uridine. The \(^{15}\text{N}-\) and \(^{17}\text{O}-\)NMR spectra of the target compounds 12 and 14 in phosphate-buffered H\(_2\)O serve as references for heteronuclear NMR spectra of labelled RNA fragments.

Introduction. — The availability of \(^{15}\text{N-}\) and \(^{13}\text{C}-\)labelled ribonucleosides are of special interest since now new NMR techniques have been established. Multidimensional heteronuclear \(^1\text{H}-\)NMR and 3D or 4D triple-resonance NMR correlation spectroscopy allow to assign overlapping \(^1\text{H}-\)NMR signals in NOESY spectra of large biomolecules such as RNA fragments through their connectivities to the often nonoverlapping \(^{15}\text{N-}\) and \(^{13}\text{C}-\)NMR signals [1]. In addition, \(^{15}\text{N}\) labels in nucleic-acid fragments are valuable solvent-nondegradable markers that can be used to monitor secondary- and tertiary-structure formation by \(^{15}\text{N}-\)NMR spectroscopy and to calculate the thermodynamics of such interactions [2] [3]. \(^{17}\text{O-}\)-Containing nucleobases show marked shifts and broadenings in their \(^{17}\text{O}-\)NMR signals upon H-bond formation and could be used as local markers in RNA fragments to confirm unusual base-base geometries by an independent method [3].

These developments formed the background to the present investigation in which the synthesis of doubly labelled pyrimidine nucleosides is described. The procedures that have been used are largely known ones. In their original forms, however, they were not suitable for the application to expensive isotopes. Our efforts aimed at reproducible experimental procedures giving high-yield products with respect to the isotope-containing precursors. In conjunction with the biosynthetic production of \(^{15}\text{N}-\)labelled purine nucleosides by an overproducing \textit{Bacillus subtilis} strain [4], this purely synthetic approach to \(^{15}\text{N}-\)labelled pyrimidine nucleosides is — without the additional steps for the introduction of the \(^{17}\text{O}\) isotopes — an alternative to the present way of obtaining \(^{15}\text{N}-\)labelled ribonucleosides from enzymatic digests of labelled RNA gained from fermentations [5].

Results and Discussion. – (\textsuperscript{15}N\textsubscript{2})/\textsuperscript{17}O\textsubscript{2}Urea. The synthetic strategy, as depicted in Scheme 1, involves the usage of a common key intermediate \textit{8a/b} for both pyrimidine nucleosides, uridine, and cytidine. Key compound \textit{8a/b} contains the two \textsuperscript{15}N isotopes of uridine and one \textsuperscript{17}O isotope in the urea-derived carbonyl group C(2)=O. The synthesis of \textit{8a/b}, therefore, necessitates the preparation of (\textsuperscript{15}N\textsubscript{2})\textsuperscript{17}O\textsubscript{2}urea (5) which is obtained from (\textsuperscript{15}N\textsubscript{2})thiourea (3) in two steps involving elimination of H\textsubscript{2}S and hydrolysis of the resulting (\textsuperscript{15}N\textsubscript{2})cyanamide (4) with H\textsubscript{2}\textsuperscript{17}O (cf. [6])\textsuperscript{3}).

Scheme 1. Synthesis of (\textsuperscript{15}N\textsubscript{2})/\textsuperscript{17}O\textsubscript{2}Uridine (12) and (\textsuperscript{15}N\textsubscript{2})/\textsuperscript{17}O\textsubscript{2}Cytidine (14)

The synthesis of thiourea was described in 1873 [7]. In analogy to the \textit{Woehler} reaction, it proceeds from thiophosgene (CS\textsubscript{2}Cl\textsubscript{2}) and, in our case, \textsuperscript{15}NH\textsubscript{4}Cl in aqueous NaOH solution to form ammonium thiocyanate 2 (\textsuperscript{15}NH\textsubscript{4}SC\textsuperscript{15}N). One equivalent of CS\textsubscript{2}Cl\textsubscript{2} consumes 2 equiv. of NH\textsubscript{4}Cl and 4 equiv. of NaOH. However, according to comprehensive descriptions of this reaction [8], several side reactions can lower the yield.

\textsuperscript{3}) For convenience, the \textsuperscript{16}O isotopes are not indicated in the formulae and in the \textit{General Part}; for accurate systematic names, see \textit{Exper. Part}.
of 2 dramatically. CSCl₂ can be hydrolyzed to CO₂, H₂S, and 2 HCl; in the presence of NH₄Cl, it can disproportionate to CS₂ and CCl₄; in the presence of O₂ and H₂O, it can be oxidized to CO(NH₂)₂ and H₂SO₄. In addition, if during workup with HCl the pH value drops below 5, thiocyanate can evaporate as volatile HSCN; in the presence of O₂, it can be oxidized to NCSSCN subsequently producing insoluble, pink polymerization products [7]. Hence, several reaction and workup conditions had to be carried out to maximize the yield of 2 with respect to consumed ¹⁵NH₄Cl.

In the optimized version, 1 equiv. of 1 was added to an aqueous, degassed solution of 2 equiv. of ¹⁵NH₄Cl at −10°C followed by 4.1 equiv. of an aqueous degassed and cooled solution of NaOH. The pH immediately rose to 13.5. During warming up to room temperature, the pH dropped to 9.2 in 1 h revealing that the reaction could not have been quantitative with respect to the consumption of ¹⁵NH₄Cl. Furthermore, the formation of insoluble polymeric brown-reddish side products ('polyrhodan') could be suppressed to some extent, but residual amounts were unavoidable. After correction of the pH value to 5.5, filtration, and evaporation, 2 was isolated by extraction with ice-cold EtOH and purified by sublimation. The excess of EtOH-insoluble ¹⁵NH₄Cl together with residual ¹⁵NH₄Cl from sublimation was recovered by placing an aqueous solution thereof into a Parnas-Wagner apparatus, adding conc. NaOH solution, and water-vapor distilling the ¹⁵NH₄Cl solution into 6n HCl. Three subsequent rounds of the described procedure resulted in chemical yields of only 55, 53, and 36% of purified 2 with respect to used 1, but 33, 35, and 52% of ¹⁵NH₄Cl, respectively, were regenerated. Thus, the total yield of purified 2 was 82% with respect to consumed ¹⁵NH₄Cl. The composition and purity of the crude and purified product was monitored by IR spectroscopy (SC¹⁵N⁻ stretching at 2039 cm⁻¹ (SC¹⁴N⁻ at 2063 ± 3 cm⁻¹)).

(¹⁵N₂)Thiourea (3) is an isomer of 2. At 160°C, the two isomers are in a 2:1 equilibrium, unfortunately, in favor of 2. The equilibrium is less favorable at lower or higher temperatures, it is reached within 2 h, and the loss of material due to the formation of H₂S and guanidinium thiocyanate [8] is only 3%, provided that the isomerization is carried out in a sealed vessel. According to a patented procedure [9], the resulting 2:1 mixture is decolorized with charcoal and a 1:1 mixture is first obtained by crystallization, owing to the better solubility of NH₂SCN in cold H₂O (addition of H₂O to the 2:1 mixture cools it down to −10°C). In a second step, pure thiourea ought to be crystallized from an aqueous solution of the 1:1 mixture.

In our hands, however, H₂O proved an unsuitable solvent. MeOH seemed better, EtOH/MeOH mixtures even more so. Again, the composition of the various fractions from the crystallizations was determined by IR spectroscopy. The intensity ratio between the mentioned absorption at ca. 2039 cm⁻¹ from 2 (ca. 2063 cm⁻¹ for unlabelled 2) and one at ca. 1580 or ca. 1481 cm⁻¹ from 3 (ca. 1591 or ca. 1468 cm⁻¹, resp., for unlabelled 3) could be used to calibrate spectra of unlabelled and determine the composition of labelled mixtures 2/3. Despite the changes in solvent composition and crystallization conditions, the difficulty of obtaining 3 with bad crystallization yields (ca. 25% per round) and only 80–95% purity remained. The problem could only be solved after the discovery that the crude 2:1 mixture can be used to directly sublime 2 out of the mixture. The residue after such a treatment contains a 1:1 mixture of 2 and 3 as a hard solid ( < 3% loss of weight). A second portion of highly pure 2 is obtained after careful powderization of the residual 1:1 mixture and resublimation. The residue then contains 3 in 95–97% chemical purity.
Nine rounds of isomerization, sublimation, and resublimation produced 3 in 66% total yield. It was used for the next step without further purification.

Yellow HgO (flavum) converts thiourea into cyanamide under virtually neutral conditions. The reaction is carried out under a layer of Et₂O containing as small amount of H₂O [10] [11]. Using these conditions, 4 was obtained from 3 in 78% yield. The melting point and IR spectrum agreed well with an unlabelled reference sample, except for the shifted nitrile stretching vibration occurring at 2229 instead of 2259 cm⁻¹ and the other stretching vibration occurring at 1569 instead of 1580 cm⁻¹.

Cyanamide is efficiently hydrolyzed to urea in 1.7N HCl during 10 min at 100°. Using this procedure, a yield of 90% was reported [12]. The molar excess of H₂O vs. cyanamide was 25:1. These conditions were unsuitable for our purpose, since we would have to use 31 ml of H₂[¹⁷O] to convert 65.5 mmol of 4 having a mere 2.87 ml to our disposal. In addition, several side reactions can occur [11]. Under slightly basic or acidic conditions, cyanamide can dimerize to dicyandiamide. Two molecules of urea can condense under exclusion of NH₃ to form biuret (iminodicarbonic diamide) which can further condense to the stable cyanuric acid. Besides, a certain extent of hydrolysis of urea to CO₂ and NH₄Cl seems unavoidable under the applied conditions.

First, the minimum amount of H₂O for a smooth conversion had to be established. Reaction conditions using 2-4N HCl with molar excesses of H₂O of 25, 11, 7, 6, 5, 2, and 1:1 revealed that, down to an excess of 5:1, satisfactory yields (75-85%) of urea could be obtained after refluxing for 25 min. No by-products were detected by IR spectroscopy. However, portions of unreacted cyanamide had to be regenerated by fractionated sublimation of the crude hydrolysate. At lower molar excesses, only dicyandiamide formed (IR).

Next, the optimal reaction time was established using a 5-fold molar excess of H₂O. Below 20 min, the crude mixture contained less than 30% of urea along with unreacted cyanamide. Above 30 min, the IR spectra began to show the formation of NH₄Cl owing to the hydrolysis of urea. Using prolonged reaction times, the yields of urea never exceeded 20%. The optimum was found to be 20–30 min reflux and neutralization with Na₂CO₃ to pH 7.0. Under these conditions, no NH₄Cl and only minimal amounts of dicyandiamide formed.

The conversion of 4 to 5 was carried out in four rounds. A small amount of concentrated HCl stock solution in H₂[¹⁷O] was used to appropriately acidify the solvent. After each round of conversion and neutralization, the excess H₂[¹⁷O] was lyophilized out of the crude mixture, to reuse it in the next round. Fortunately, 4 sublimed during 10–20 min between 50 and 75° at 25 μbar, whereas 5 needed 110–120° and 2–3 h. After each round, 10, 14, 24, and 0% of 4 were regenerated and reused. The total yield of sublimed 5 was 75% and 46.5% of H₂[¹⁷O] could be regenerated at the end. Less than 3% of biuret was found in the residue after sublimation. The mass spectra of each portion showed signals at m/z 62, 63, and 64 corresponding to the (¹⁵N₂,¹⁶O)−, (¹⁵N₂,¹⁷O)−, and (¹⁵N₂,¹⁸O)-isotopomers of urea, respectively. The intensity of the signals revealed an isotope dilution of ca. 2.8 atom-% ¹⁶O per round owing to the successive addition of unlabelled Na₂CO₃ (without neutralization, the oily hygroscopic hydrochloride of urea was obtained even after resublimation). The average O-isotope composition corresponded to the one of H₂[¹⁷O]; 36 atom-% ¹⁸O, 35 atom-% ¹⁷O, 29 atom-% ¹⁶O.
In the presence of polyphosphoric acid, urea reacts with propynoic acid to form uracil \([13]\). The reaction of 5 proceeded smoothly at 85°C. After workup, a major part of \((^{15}N_2)[O^{2-17}O]\)uracil (6) crystallized from the crude mixture. It was recrystallized from H$_2$O. More 6 resulted from resublimation and extraction of the evaporated mother liquors. The combined yield of pure 6 was 76%.

Nucleoside derivative 7 was obtained from ribosylation of 6 under Vorbrüggen conditions \([14]\). A small portion of 7 was debenzoylated to give \((^{15}N_2)[O^{2-17}O]\)uridine; its $^{17}$O-NMR spectrum in phosphate-buffered H$_2$O (pH 7.0, 45°C) shows a signal at 239.7 ppm \((
u = 650 \text{ Hz})\) relative to external 1,4-dioxane (not shown$^4$).

Activation of unlabelled 7, i.e., u-7, with 3-nitro-1H-1,2,4-triazole and diphenylphosphorochloridate, a method that was so efficient for the corresponding activation of thymine derivatives \([6, 15]\), merely resulted in the formation of \((2',3',5'-\text{tri-O-benzoyl-uridin-04-yl})\) 3-nitro-1H-1,2,4-triazolyl phenyl phosphate (30% yield), a dead-end product with respect to further substitutions at O$_4$. In contrast, the relatively stable crystalline key intermediate u-8a/b was obtained from u-7 in 94% yield using phosphoryltribis(1H-1,2,4-triazole) as activator \([16]\). Despite the narrow melting range (181.5–183.3°C), a HETCOR spectrum showed u-8a/b to occur as a ca. 1:1 mixture of two regioisomers, the 1,2,4-triazol-1-yl (u-8a) and the -4-yl (u-8b) derivatives.

During our attempts to hydrolyze u-8a/b with a minimal amount of water, we observed once more a marked difference between the reactivity of an activated thymine derivative (a 4-dehydroxy-5-methyl-4-(3-nitro-1H-1,2,4-triazolyl)uridine derivative \([6]\))

\[
\text{DBU as a Nucleophile}
\]

\[u-8a/b \xrightarrow{\text{DBU}} u-9 \xrightarrow{\text{CH$_3$ONa}} u-12 \]

\[R = \text{benzoyl, H}
\]

In one case, a side product due to over-ribosylation occurred. After debenzoylation, it was identified as 3-(β-D-ribofuranosyl)-\((^{15}N_2)[O^{2-17}O]\)uridine by \(^1H\), \(^13C\), \(^15N\), and \(^17O\)-NMR and FAB-MS (pos.). The \(^15N\)-NMR spectrum in aqueous sodium phosphate buffer at pH 7.0 shows two $d_2$'s \((J = 2.3 \text{ Hz})\) at 124.0 (N(1)) and 150.3 ppm \((
u = 1700 \text{ Hz})\). In the \(^17O\)-NMR spectrum under the usual conditions, a signal appears at 263.6 ppm \((
u = 1700 \text{ Hz})\). The FAB-MS (pos.; glycerine/H$_2$O) shows 3 groups of signals at $m/z$: 381/380/379 \((M^+)\), 249/248/247 \((\text{[M – ribosyl]}^+)\), and 117/116/115 \((\text{[M – 2 ribosyl]}^+)\). This compound could be converted to \((^{15}N_2)[O^{2-17}O]\)uridine by treatment with 12N HCl at room temperature for 24 h. For experimental details, see Footnote 1.
and uridine derivative u-8a/b. While the former would smoothly hydrolyze in the presence of 1.9 equiv. of H_2O and 1.6 equiv. of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), the latter needed 5 equiv. of both reagents for complete conversion. TLC Analysis showed that the benzoyl groups were slowly cleaved during the hydrolysis. The decreased reactivity of u-8a/b does not surprise. However, the fact that, after a subsequent methanolysis of

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**Figure.** 1^n-O-NMR Spectrum of a) 12 and b) 14. In 0.1m sodium phosphate, pH 7.0 at 45°; external standard 1,4-dioxane (0 ppm).
the residual benzoyl groups, only 31% of uridine was isolated, together with 14% of 
$O^\cdot$-methyluridine ($u$-$10$), and 31% of another compound that was identified as DBU substitution product $u$-$11$, suggests a more complicated mechanism than initially ex-
pected (Scheme 2).

Apparently, the prolonged reaction time allowed for a nucleophilic attack of unre-
acted starting material by freshly formed, deprotonated $2',3',5'$-tri-$O$-benzoyluridine
resulting in considerable amounts of 'dimeric' compound $u$-$9$ (TLC). During the sub-
sequent in situ methanolysis, all compounds debenzoylated. TLC confirmed the for-
amation of uridine and presumably debenzyolated 'dimer$^\cdot$'. Part of $u$-$9$ underwent a substitu-
tion by methanolate to give $u$-$10$ and $u$-$12$. In addition, a nucleophilic substitution by
DBU took place during the evaporation of the solvents (TLC) confirming its recently
reported dual role as base and nucleophile [17]. According to the mechanism proposed by
Lammers et al., the primary cationic DBU adduct hydrolyzed and rearranged to form the
ring-opened product $u$-$11$.

The problem was circumvented by using aqueous Na$[^{17}$O]$\text{H}$ solution in THF for the
hydrolysis of $8a/b^5)$. The melting range and the spectroscopic data of $12$ ($^1$$H$, $^1$$C$, $^1$$N$,,$^1$$O$-NMR and FAB-MS (pos.)) confirmed its purity and structure ($^1$$N$-NMR: 125.0 (N(1)) and 138.4 ppm (N(3)), $J$(N,N)'s not resolved). The $^1$$O$-NMR spectrum of $12$ is depicted in the Figure.

($^{15}$N)$_2$/O$^{17}$O/Cytidine (14). The ammonolysis of $8a/b$ appeared much more
straightforward than its hydrolysis (Scheme 1). An oily saturated THF solution of $8a/b$
was slowly added to aqueous ca. 25% $^{15}$NH$_2$OH solution. Tribenzoylcytidine $13$
precipitated almost instantaneously. Workup and chromatographic purification resulted in
a recovery of ca. 80% of the used $^{15}$NH$_2$ and 5.6% of unreacted $8a/b$ and in a 71 % yield of
pure $13$. A fraction of $13$ was subsequently deprotected in 28% NH$_2$OH/THF for 2 days
at 55o to give pure $14^6)$. The melting range and the spectroscopic data of $14$ ($^{15}$N- and
$^{17}$O-NMR, FAB-MS (pos.)) confirmed its purity and structure ($^{15}$N-NMR: 72.2 (N$^\cdot$),
131.6 (N(1)), and 181.4 ppm (N(3)), $J$(N,N)'s not resolved). The $^{17}$O-NMR spectrum of $14$
is depicted in the Figure.

The financial support from the Swiss National Science Foundation is gratefully acknowledged.

Experimental Part

**General.** See [6]. Moreover or differing from it: ($^{15}$N)$_2$Urea (99 atom-% $^{15}$N) was purchased from Cambridge
Isotope Laboratories, H$_2[^{17}$O] (3 g; 36 atom-% $^{16}$O, 35 atom-% $^{17}$O, 29 atom-% $^{18}$O, normalized) from Iso-Yeda
Co. Ltd., Rehovot, Israel. Less enriched H$_2[^{17}$O] (2.3 ml, 21 atom-% $^{17}$O) was a generous gift from Prof. Hans
Dahn, Institut de Chimie Organique, Universitié de Lausanne. Medium-pressure liquid chromatography (MPLC):
max. 40 bar; Büchi; programmed low-pressure gradient mixing, UV detection with prep. flow-cell and variable
wavelength (Kontron); reversed-phase column (Büchi): 46 × 3.6 cm (i.d.) containing 250 g of LiChroprep$^\text{b}$ RP-18
(Merck), 15-25 μm (packed as toluene suspension). M.p.: Køfer block with digital temp. display; corrected.

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5) Although the yields in preliminary experiments with $u$-$8a/b$ consistently resulted in 76% of pure uridine, the
crucial experiment with labelled $8a/b$ afforded $12$ in 40% yield. The unexpected loss occurred during MPLC
purification, see Exper. Part.

6) We shall acylate the remaining amount of $13$ on N$^\cdot$ so that it can be further converted into a DNA/RNA-syn-
thesizer-compatible phosphoramidite.
IR Spectra: Perkin-Elmer-1600-FTIR spectrometer, Hewlett-Packard Color Pro plotter; v in cm⁻¹. MS: B = nucleobase fragment. Elemental analysis: the ¹⁵N/¹⁴N-correction factor was determined to be insignificant (1.0065 using solid ¹⁵NH₄Cl and ¹⁴NH₂Cl).

(¹⁵N)Ammonium (¹⁵N)Thiocyanate (2). A soln. of ¹⁵NH₄Cl (19.928 g, 366 mmol) in H₂O (250 ml; ultrasonically degassed) was cooled down to −10° in a 500-ml flask equipped with an Ar/dropping-funnel inlet, pH electrode, and outlet serially connected to 2 washing flasks, the latter containing 6N HCl. First, CSCI₂ (1: 14 ml, 185 mmol; Aldrich), then, a separately degassed and cooled (0°) NaOH soln. (29.8 g, 745 mmol) in H₂O (60 ml) were steadily added to the ¹⁵NH₄Cl soln. under an Ar flow and without stirring (CSCI₂ is light and H₂O-immiscible; conc. NaOH forms a layer at the bottom of the flask). The Ar flow was stopped, and rapid stirring was switched on. The pH rose immediately to 13.5 and dropped continuously to 9.2 within 1 h. A ‘rusty’ precipitate formed. The pH value was corrected with a few drops of conc. HCl to 5.5. The mixture was filtered, the precipitate washed with ice-cold EtOH (4 × 100 ml), the filtrate evaporated, and the residue dried under high vacuum (h.v.) overnight: 9.22 g of crude 2. Resublimation (120°/50 µbar, 3 h) furnished 7.871 g of pure, slightly yellowish 2 (201 mVal ¹⁵N, 55.1% rel. to used ¹⁵NH₄Cl; 2nd round: 2.509 g (53.3%); 3rd round: 0.592 g (36.2%)). Total yield after 4 rounds: 10.972 g of 2 (281 mVal ¹⁵N, 81.6% rel. to consumed ¹⁵NH₄Cl). M. p. 140–150°. IR (BrKb): 3450s (br. sh), 3123s (br.), 2039s, 1624~1, 1394s.

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(¹⁵N)Ammonium (¹⁵N)Thiocyanate (2). A soln. of ¹⁵NH₄Cl (19.928 g, 366 mmol) in H₂O (250 ml; ultrasonically degassed) was cooled down to −10° in a 500-ml flask equipped with an Ar/dropping-funnel inlet, pH electrode, and outlet serially connected to 2 washing flasks, the latter containing 6N HCl. First, CSCI₂ (1: 14 ml, 185 mmol; Aldrich), then, a separately degassed and cooled (0°) NaOH soln. (29.8 g, 745 mmol) in H₂O (60 ml) were steadily added to the ¹⁵NH₄Cl soln. under an Ar flow and without stirring (CSCI₂ is light and H₂O-immiscible; conc. NaOH forms a layer at the bottom of the flask). The Ar flow was stopped, and rapid stirring was switched on. The pH rose immediately to 13.5 and dropped continuously to 9.2 within 1 h. A ‘rusty’ precipitate formed. The pH value was corrected with a few drops of conc. HCl to 5.5. The mixture was filtered, the precipitate washed with ice-cold EtOH (4 × 100 ml), the filtrate evaporated, and the residue dried under high vacuum (h.v.) overnight: 9.22 g of crude 2. Resublimation (120°/50 µbar, 3 h) furnished 7.871 g of pure, slightly yellowish 2 (201 mVal ¹⁵N, 55.1% rel. to used ¹⁵NH₄Cl; 2nd round: 2.509 g (53.3%); 3rd round: 0.592 g (36.2%)). Total yield after 4 rounds: 10.972 g of 2 (281 mVal ¹⁵N, 81.6% rel. to consumed ¹⁵NH₄Cl). M. p. 140–150°. IR (BrKb): 3450s (br. sh), 3123s (br.), 2039s, 1624~1, 1394s.

IR Spectra: Hewlett-Packard Color Pro plotter;  v in cm⁻¹. MS: B = nucleobase fragment. Elemental analysis: the ¹⁵N/¹⁴N-correction factor was determined to be insignificant (1.0065 using solid ¹⁵NH₄Cl and ¹⁴NH₂Cl).
48.8, $[^{15}N]J / O_2^{17}/O_{18}$]/[Uracid (6). A mechanically stirred suspension of 5 (2.895 g, 45.97 mmol), polyphosphoric acid (80 g) and freshly distilled propionic acid (3.1 ml, 53.3 g, 50.4 mmol) was heated to 89°C. After 15–20 min, the initially colorless suspension turned orange. After 4 h, the mixture was removed from the oil bath, and stirring was continued for 16 h at r.t. The brown paste was hydrolyzed with H$_2$O (160 ml). The resulting light-yellow so. was stirred for 1 h at r.t. and then cooled down to –10°C to crystallize crude 6. The mixture was neutralized with 6 N NaOH and the suspension cooled and filtered. The turbid orange mother liquor was concentrated to 1/3 of its volume to crystallize a 2nd portion of crude 6. The combined portions were recrystallized in hot H$_2$O: 3.08 g (58%) of TLC-pure yellowish solid (TLC: AcOEt/MEOH/H$_2$O 4:1:2: Rf 0.5). The 2nd mother liquor was evaporated and lyophilized and the residue sublimed (150–200°C, 5 μbar, +10°C) to give 0.70 g (13%) of TLC-pure colorless 6. The residue from sublimation was continuously extracted with AcOEt to give 0.25 g (4.7%) of TLC-pure orange solid: 4.03 g (76%) of 6 as a pale-yellow solid. $^1$H-NMR (300 MHz, CD$_2$SOCD$_3$, SiMe$_4$): 5.46 (dt, $^3$J(H-C(6), H-C(5)) = 7.5, $^2$J(N(1), H-C(5)) = 8.0, J(N(3), H-C(5)) = 7.5, H-C(5)); 7.39 (dt, $^2$J(H-C(5), H-C(6)) = 7.5, $^2$J(N(3), H-C(6)) = 7.5, H-C(6)); 10.9, 11.2 (br., H-N(3), H-N(1)). EIMS (70 eV): 116 (48.2, [$(^{16}O, ^{16}O)M^+]$), 115 (58.4, [$(^{15}O, ^{15}O)M^+]$), 114 (61.7, [$(^{16}O)M^+]$). Anal. calc. for C$_{18}$H$_{23}$N$_3$O$_4$SiMe$_4$: C 55.7, H 4.8, N 10.06, %.

2',3',5'-Tri-O-benzoyl-$[^{15}N]J / O_2^{17}/O_{18}$]/[Juridine (7) was synthesized according to [14a] using equimolar amounts of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-ribofuranose and SnCl$_4$ in CH$_2$CH$_2$Cl at r.t. Pure 7 was obtained after CC (SiO$_2$, 2% MEOH/CH$_2$Cl$_2$, SiMe$_4$): 4.68 (dt, $^3$J(H-C(6), H-C(5)) = 7.5, $^2$J(N(1), H-C(5)) = 8.0, $^2$J(N(3), H-C(5)) = 7.5, H-C(5)); 7.39 (dt, $^2$J(H-C(5), H-C(6)) = 7.5, J(N(3), H-C(6)) = 7.5, H-C(6)); 10.9, 11.2 (br., H-N(3), H-N(1)). EIMS (70 eV): 116 (48.2, [$(^{16}O, ^{16}O)M^+]$), 115 (58.4, [$(^{15}O, ^{15}O)M^+]$), 114 (61.7, [$(^{16}O)M^+]$). Anal. calc. for C$_{35}$H$_{41}$N$_5$O$_9$SiMe$_4$: C 71.4 (C(5)), 71.72 (C(2')), 90.51 C(1'), 88.09 (d, J(N(1), C(1)')) = 13.8, C(1')); 103.40 (d, $^2$J(N(1), C(5)) = 7, C(5)); 128–130 (C$_6$C$_5$M$_{-}$C$_{6}$); 133.66, 133.76, 133.82 (C$_{phen}$); 139.56 (d, J(N(1), C(6)) = 12.6 C(6)); 149.93 (s, J(N(3), C(2')) = J(N(3), C(2)) = 18, C(2')); 162.50 (d, J(N(3), C(4)) = 9.2, C(4')); 165.29, 165.34, 166.05 (PhCO).

4-Dehydroxy-4'-1'H-1',2',4'-triazol-1'-l/(and 4'-l)/2',3',5'-tri-O-benzoyl-$[^{15}N]J / O_2^{17}/O_{18}$]/[Juridine (8a/b). To a vigorously stirred suspension of 1H-1,2,4-triazole (10.698 g, 154.68 mmol) in dry MeCN (140 ml), POCI$_3$ (3.48 ml, 38.16 mmol) was added at –10°C during 2–3 min, followed by Et$_3$N (24.8 ml, 177.37 mmol) during 5 min. After 1 h at –5 to 0°C, a soln. of 5.77 g (10.31 mmol) of 7 in dry MeCN (100 ml) was added and the mixture stirred at r.t. overnight. The orange suspension was rapidly filtered through SiO$_2$ (200 g: h 8 cm, $\phi$ 9 cm; MeCN), the filtrate evaporated, and the mixture submitted to FC (SiO$_2$ (250 g), MeCN; Rf 0.6); 5.92 g (94%) of 8a/b. Yellow solid. m.p. 180–183°C. $^1$H-NMR (300 MHz, CDCl$_3$, SiMe$_4$): 4.75 (dd, $^3$J = 2, $^3$J = 12, H$_2$-C(5)); 4.85 (m, $^3$J(H-C(6), H-C(5)) = 7.5, H-C(4)); 4.90 (dd, $^3$J = 2, $^2$J = 12, H$_2$-C(5)); 5.86 (dd, $^3$J(H-C(6), H-C(5)) = 4.5, $^3$J(N(1), H-C(6)) = 1.5, H-C(6)); 5.93 (t, $^3$J(H-C(2'), H-C(3')) = 1.5, H-C(2')); 6.27 (t, $^3$J(H-C(2'), H-C(3')) = 1.5, H-C(3')); 6.64 (d, $^3$J(H-C(2'), H-C(3')) = 4.6, H-C(1')); 6.96 (dt, $^3$J(H-C(6), H-C(5)) = 7.4, $^3$J(N(3), H-C(5)) = 7.4, H-C(5)); 7.27–7.64, 7.95–8.09, 8.11–8.12 (3m, H-C(6), 3 Ph). 8.19, 8.20 (5s, H-C(3'), H-C(5')).

O'^4-Methyluridine (u-10). N$_4$'-5'-[(Hexahydro-2-oxo-1H-azepin-1'-yl)propyllcytidine (u-11) and Uridine (u-12). A soln. of 8a/b (1.377 g, 2.27 mmol) in H$_2$O (163.3 μl, 9.07 mmol) was added, and after a total reaction time of 25 h, THF was evaporated, CHCl$_3$ added, and the soln. extracted withaq. NaHCO$_3$ soln. (2 x ) and H$_2$O (5 x), dried (Na$_2$SO$_4$), and evaporated. The dark orange foam was dissolved in abs. MeOH and co-evaporated 5 x before addition of abs. MeOH (10 ml) and 0.353N NaOMe/MeOH (20 ml). After 45 min at r.t., the soln. was neutralized with Dowex 50W x 8 (20 50 mesh, H$^+$ form), filtered, and evaporated. The crude solid was dissolved in H$_2$O and theaq. soln. extracted with CHCl$_3$ and lyophilized: 581 mg. Purification by FC (SiO$_2$ (30 g), AcOEt/MeOH/H$_2$O 4:1:0.2) furnished 253 mg of a 2:1 mixture (1'H-NMR; R$^f$ 0.5) of u-12 (31%) and u-10 (14%) and 278 mg (31%) of u-11 (R$^f$ 0.3).
u-12/u-10 2:1; 1H-NMR (300 MHz, CD3SOCD3, SiMe4): 3.56 (add, 3J(H,-CH2(5')) = 5.0, 3J = 12, 3J = 3.7, H3-C(5'), 12, 12, 10); 3.63 (add, 3J(3',OH,CH2(5')) = 5.0, 3J = 12, 3J = 3.3, H3-C(5), u-12, u-10); 3.83 (s, MeO-C(4), u-10); 3.85 (dd, 3J(H,-C(3'),OH-C(3')) = 4.3, OH-C(3'), 12, 10); 5.09 (m, OH-C(5'), OH-C(3'), 12, 10); 5.15 (t, 3J(H3-C(5'),OH-C(5'), 12, 10); 5.37 (d, 3J(H3-C(2'),OH-C(2')) = 5.4, OH-C(2'), 12, 10); 5.45 (d, 3J(H3-C(5),OH-C(6)) = 8.2, H-C(6), 12, 10); 7.89 (dd, 3J(H3-C(2'),H-C(6)) = 5.4, H-C(1'), 12, 10); 6.05 (d, 3J(H3-C(6), H-C(5)) = 7.4, H-C(5), 10, 10); 7.19 (d, 3J(H3-C(5),H-C(6)) = 8.2, H-C(6), 12, 12, 8.32 (d, 3J(H3-C(5),H-C(6)) = 7.4, H-C(6), 10, 11.29 (br. s, H-N(3), 13); 13C-NMR (75 MHz, CD3SOCD3, SiMe4): 45.42 (MeO-C(4), u-10), 60.55 (C(5'), u-10); 61.33 (C(5), u-12); 69.37 (C(3'), u-10); 70.35 (C(3'), u-12); 73.97 (C(2'), u-10); 74.90 (C(2'), u-10); 84.71 (C(4'), u-10); 85.33 (C(4'), u-12); 88.25 (C(1'), 12); 90.36 (C(1'), u-10); 95.46 (C(5), u-10); 102.37 (C(6), u-12); 141.45 (C(6), u-12); 144.83 (C(2), u-12); 151.27 (C(2), u-12); 153.79 (C(2), u-10), 163.92 (C(4), u-12); 171.80 (C(4), u-10). FAB-MS (pos., nitrobenzyl alcohol): 259 (51.7 [M + H]+, u-10), 245 (31.9, [M + H]+, u-12), 127 (100, [B + 2H]+, u-10), 113 (41.8, [B + 2H]+, u-12), 89 (10.6), 79 (9.8).

u-11: 1H-NMR (300 MHz, CD3SOCD3, SiMe4): 1.62 (m, 8 H, 4 × CH2); 2.42 (m, 2 H, CH2); 3.23 (t, 3J = 7, 2 H, CH2); 3.33 (m, 4 H, CH2); 3.56 (d, 3J(H3-C(5'),H3-C(5')) = 12, H3-C(5'), 3J = 3.66 (d, 3J=2H3-C(5'), H3-C(5')) = 12, H3-C(5'), 3.82 (br. H, C(4')); 5.00 (br. s, OH-C(3'), OH-C(3')); 5.28 (br. s, OH-C(2')); 5.72 (d, 3J(H3-C(6),H3-C(5')) = 7.4, H-C(5), 3J = 5.76 (d, 3J(H3-C(2'),H-C(1')) = 3.2, 3J=2H3-C(2'), H-C(1')); 7.65 (t, 3J=2H3-C(2'), H-C(1')); 7.86 (add, 3J(H-C(2'),H-C(1')) = 12, H-C(1'), 3J = 5.3, H-C(1'); 7.88 (add, 3J(H-C(5),H-C(6)) = 8, J(N(1),H-C(6)) = 2, H-C(6); ca. 11.3 (br. s, H,N(3)); 13C-NMR (101 MHz, CD3SOCD3, SiMe4): 60.81 (C(5')); 69.85 (C(3')); 73.47 (C(2')); 84.82 (C(4')); 87.69 (d, 3J(N(1),C(1)) = 12.2, C(1')); 101.80 (d, 3J(N',C(5)) = 6.4, C(5')); 146.78 (d, 3J(N(1),C(6)) = 12.2, C(6)); 150.83 (t, 3J=J(N(1),C(2)) = 1,J(N(1),C(2)) = 17.5, C(2)); 163.17 (d, 3J=J(N(3),C(4)) = 9.3, C(4)); 13N-NMR (41 MHz, H3;D-band-decoupled, 0.1m acq, sodium phosphate buffer, pH 7.0, 5% (v/v) D2O, 25°, internal 15NH4Cl); 124.98 (br. s, 3J(N(1),3J(N(1),C(1))) = 138.42 (br. s, N(3')); 170-NMR (54 MHz, 0.1m acq, sodium phosphate buffer, pH 7.0, 45°, external 1,4-dioxane (neat)); 238.9 (br. s, w/v ≈ 690°, O-C(2)); 297.9 (br. s, w/v ≈ 740°, O-C(4)). FAB-MS (pos., glycercine/H2O): 252 (4.0, [15O2]O2 + 2H2), 251 (20.5, [15O2]O2 + 2H2 + [15O2]O2 + M + H+), 250 (43.4, [15O2]O2 + 2H2 + [15O2]O2 + 2H2 + [15O2]O2 + M + H+), 249 (91.2, [15O2]O2 + 2H2 + [15O2]O2 + 2H2 + [15O2]O2 + M + H+), 248 (85.7, [15O2]O2 + 2H2 + [15O2]O2 + 2H2 + [15O2]O2 + M + H+), 247 (69.9, [15O2]O2 + M + H+), 246 (63.9, 120 (7), [15O2]O2 + 2H2), 119 (16.3, [15O2]O2 + 2H2 + [15O2]O2 + M + H+), 118 (35.6, [15O2]O2 + 2H2 + [15O2]O2 + 2H2 + [15O2]O2 + M + H+), 117 (100.0, [15O2]O2 + 2H2 + [15O2]O2 + M + H+), 116 (95.2, [15O2]O2 + 2H2 + [15O2]O2 + M + H+), 97 (16.8), 73 (23.2).

1) δ (ext. neat Me3NO3 = 360.7 ppm + δ (int. qaq. 15NH4Cl).
2) Signal width [Hz] at half the signal intensity.
2',3',5'-Tri-O-benzoyl-[15N3]/[O2,17/18O]cytidine (13). A solution of 8a/b (3.6 g, 5.9 mmol) in THF (5 ml) was slowly added into a sealed flask containing a stirred ca. 25% [15]NH4OH soln. (ca. 12 ml) that was prepared by adding 30 ml of an aq. soln. containing [15]NH4Cl (10 g, 183.5 mmol) to a conc. soln. containing NaOH (97 g) and H2O (97 ml), heating it with an electric fan, and capturing the [15]NH3 gas through a distillation bridge into a cooled flask (liquid N2) containing H2O (8.16 ml). Immediately after addition of 8a/b, an orange precipitate formed. After stirring for 5 h at r.t., TLC (AcOEt/MeOH/H2O 4:1.0.2) showed almost complete conversion to 13 (Rf 0.7) with no sign of 8a/b. The liquid was pipetted into a separate flask which, as described above, was connected to a cooled flask containing H2O (6.4 ml), and was heated to regenerate 2.56 g (Rf 0.7) with no sign of 8a/b. The crude product (2.79 g) was purified by FPC (SiO2, 135 g, 0–30% MeOH/MeCN) to give 0.2 g (5.6%) of pure 8a/b and 2.21 g (71%) of 13. 1H-NMR (300 MHz, CDCl3, SiMe4): δ 3.71 (C(5')); 71.04 (79.7, [('O1)B

References