

24. Synthesis of ($^{15}\text{N}_2$)[^{17}O]Urea, ($^{15}\text{N}_2$)[O^2, O^4 - $^{17}\text{O}_2$]Uridine, and ($^{15}\text{N}_3$)[O^2 - ^{17}O]Cytidine

by Antonio Amantea¹), Matthias Henz²), and Peter Strazewski*

Institut für Organische Chemie der Universität Basel, St. Johannis-Ring 19, CH-4056 Basel

(21.IX.95)

A general synthetic approach for the synthesis of ^{15}N - and ^{17}O -doubly labelled pyrimidine nucleosides is described. The ^{15}N isotopes in uridine and the ^{17}O isotope in the urea-derived carbonyl group of uridine and cytidine originate from ($^{15}\text{N}_2$)[^{17}O]urea (**5**) which was synthesized from $^{15}\text{NH}_4\text{Cl}$, thiophosgene (**1**), and H_2 [^{17}O]. The third ^{15}N isotope of cytidine in 4-position stems from the substitution of the 1,2,4-triazole moiety of ($^{15}\text{N}_2$)[$\text{O}^2,^{17}\text{O}$]uridine derivative **8a/b** with $^{15}\text{NH}_4\text{OH}$. Hydrolysis of the same key intermediate **8a/b** with $\text{Na}[^{17}\text{O}]\text{H}/\text{H}_2$ [^{17}O] introduced the second ^{17}O isotope into the 4-position of uridine. The ^{15}N - and ^{17}O -NMR spectra of the target compounds **12** and **14** in phosphate-buffered H_2O serve as references for heteronuclear NMR spectra of labelled RNA fragments.

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

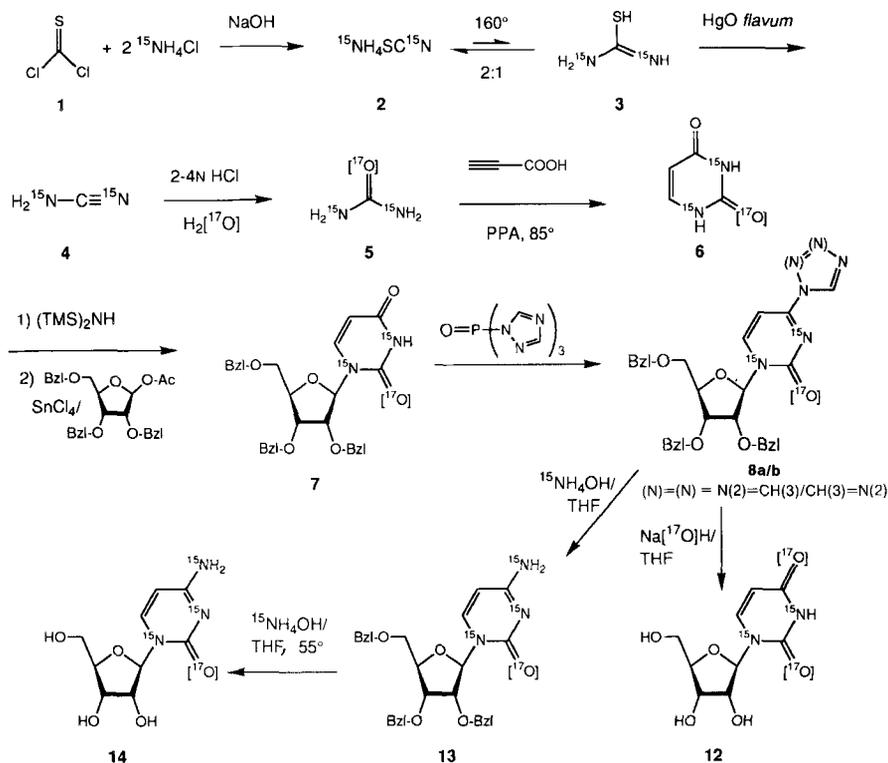
These developments formed the background to the present investigation in which the synthesis of doubly labelled pyrimidine nucleosides is described. The procedures that have been used are largely known ones. In their original forms, however, they were not suitable for the application to expensive isotopes. 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}N -labelled purine nucleosides by an overproducing *Bacillus subtilis* strain [4], this purely synthetic approach to ^{15}N -labelled pyrimidine nucleosides is – without the additional steps for the introduction of the ^{17}O isotopes – an alternative to the present way of obtaining ^{15}N -labelled ribonucleosides from enzymatic digests of labelled RNA gained from fermentations [5].

¹) Part of the Ph. D. Thesis of A. A., University of Basel, 1995.

²) Diploma Thesis, University of Basel, 1992.

Results and Discussion. – ($^{15}\text{N}_2$)[^{17}O]Urea. The synthetic strategy, as depicted in *Scheme 1*, involves the usage of a common key intermediate **8a/b** for both pyrimidine nucleosides, uridine, and cytidine. Key compound **8a/b** contains the two ^{15}N isotopes of uridine and one ^{17}O isotope in the urea-derived carbonyl group $\text{C}(2)=\text{O}$. The synthesis of **8a/b**, therefore, necessitates the preparation of ($^{15}\text{N}_2$)[^{17}O]urea (**5**) which is obtained from ($^{15}\text{N}_2$)thiourea (**3**) in two steps involving elimination of H_2S and hydrolysis of the resulting ($^{15}\text{N}_2$)cyanamide (**4**) with H_2 [^{17}O] (*cf.* [6]³).

Scheme 1. Synthesis of ($^{15}\text{N}_2$)[O^2, O^4 - $^{17}\text{O}_2$]Uridine (**12**) and ($^{15}\text{N}_3$)[O^2 - ^{17}O]Cytidine (**14**)



PPA = polyphosphoric acid, TMS = trimethylsilyl, Bzl = benzoyl, Ac = acetyl,
DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, THF = tetrahydrofuran.

The synthesis of thiourea was described in 1873 [7]. In analogy to the *Woehler* reaction, it proceeds from thiophosgene (CSCl_2 ; **1**) and, in our case, $^{15}\text{NH}_4\text{Cl}$ in aqueous NaOH solution to form ammonium thiocyanate **2** ($^{15}\text{NH}_4\text{SC}^{15}\text{N}$). One equivalent of CSCl_2 consumes 2 equiv. of NH_4Cl and 4 equiv. of NaOH. However, according to comprehensive descriptions of this reaction [8], several side reactions can lower the yield

³) For convenience, the ^{18}O isotopes are not indicated in the formulae and in the *General Part*; for accurate systematic names, see *Exper. Part*.

of **2** dramatically. CSCl_2 can be hydrolyzed to CO_2 , H_2S , and 2HCl ; in the presence of NH_4Cl , it can disproportionate to CS_2 and CCl_4 ; in the presence of O_2 and H_2O , it can be oxidized to $\text{CO}(\text{NH}_2)_2$ and H_2SO_4 . In addition, if during workup with HCl the pH value drops below 5, thiocyanate can evaporate as volatile HSCN ; in the presence of O_2 , 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 $^{15}\text{NH}_4\text{Cl}$.

In the optimized version, 1 equiv. of **1** was added to an aqueous, degassed solution of 2 equiv. of $^{15}\text{NH}_4\text{Cl}$ at -10° 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 $^{15}\text{NH}_4\text{Cl}$. 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 $^{15}\text{NH}_4\text{Cl}$ together with residual $^{15}\text{NH}_4\text{Cl}$ from sublimation was recovered by placing an aqueous solution thereof into a *Parnas-Wagner* apparatus, adding conc. NaOH solution, and water-vapor distilling the $^{15}\text{NH}_3$ 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 $^{15}\text{NH}_4\text{Cl}$, respectively, were regenerated. Thus, the total yield of purified **2** was 82% with respect to consumed $^{15}\text{NH}_4\text{Cl}$. The composition and purity of the crude and purified product was monitored by IR spectroscopy (SC^{15}N^- stretching at $2039 \pm 1 \text{ cm}^{-1}$ (SC^{14}N^- at $2063 \pm 3 \text{ cm}^{-1}$)).

($^{15}\text{N}_2$)Thiourea (**3**) is an isomer of **2**. At 160° , 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 H_2S 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 NH_4SCN in cold H_2O (addition of H_2O to the 2:1 mixture cools it down to -10°). In a second step, pure thiourea ought to be crystallized from an aqueous solution of the 1:1 mixture.

In our hands, however, H_2O 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 dicyanodiamide. 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 dicyanodiamide 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 dicyanodiamide 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-% ¹⁶O, 35 atom-% ¹⁷O, 29 atom-% ¹⁸O.

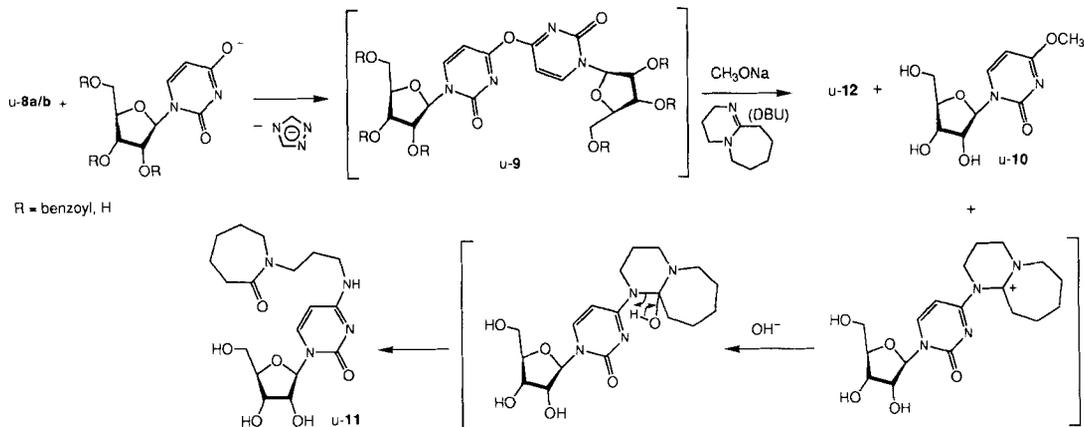
($^{15}\text{N}_2$)[O^2, O^4 - ^{17}O]Uridine (**12**). In the presence of polyphosphoric acid, urea reacts with propynoic acid to form uracil [13]. The reaction of **5** proceeded smoothly at 85°. After workup, a major part of ($^{15}\text{N}_2$)[O^2 - ^{17}O]uracil (**6**) crystallized from the crude mixture. It was recrystallized from H_2O . 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 ($^{15}\text{N}_2$)[O^2 - ^{17}O]uridine; its ^{17}O -NMR spectrum in phosphate-buffered H_2O (pH 7.0, 45°) shows a signal at 239.7 ppm ($w_{1/2} = 650$ Hz) relative to external 1,4-dioxane (not shown)⁴.

Activation of unlabelled **7**, *i.e.*, u-**7**, with 3-nitro-1*H*-1,2,4-triazole and diphenyl phosphorochloridate, a method that was so efficient for the corresponding activation of thymine derivatives [6] [15], merely resulted in the formation of (2',3',5'-tri-*O*-benzoyl-uridin-*O}^4*-yl) 3-nitro-1*H*-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 phosphor-yltris(1*H*-1,2,4-triazole) as activator [16]. Despite the narrow melting range (181.5–183.3°), 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-1*H*-1,2,4-triazolyl)uridine derivative [6])

Scheme 2. DBU as a Nucleophile



⁴) In one case, a side product due to over-ribosylation occurred. After debenzoylation, it was identified as 3-(β -D-ribofuranosyl)-($^{15}\text{N}_2$)[$\text{O}^2, ^{17}\text{O}$]uridine by ^1H -, ^{13}C -, ^{15}N -, and ^{17}O -NMR and FAB-MS (pos.). The ^{15}N -NMR spectrum in aqueous sodium phosphate buffer at pH 7.0 shows two *d*'s ($J = 2.3$ Hz) at 124.0 (N(1)) and 150.3 ppm (N(3); rel. to internal $^{15}\text{NH}_4\text{Cl}$). In the ^{17}O -NMR spectrum under the usual conditions, a signal appears at 263.6 ppm ($w_{1/2} \approx 1700$ Hz). The FAB-MS (pos.; glycerine/ H_2O) shows 3 groups of signals at m/z 381/380/379 (M^+), 249/248/247 ($[M - \text{ribose}]^+ = [\text{uridine}]^+$), and 117/116/115 ($[M - 2 \text{ ribosyl}]^+ = [\text{uracil}]^+$). This compound could be converted to ($^{15}\text{N}_2$)[$\text{O}^2, ^{17}\text{O}$]uridine by treatment with ^{12}N 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

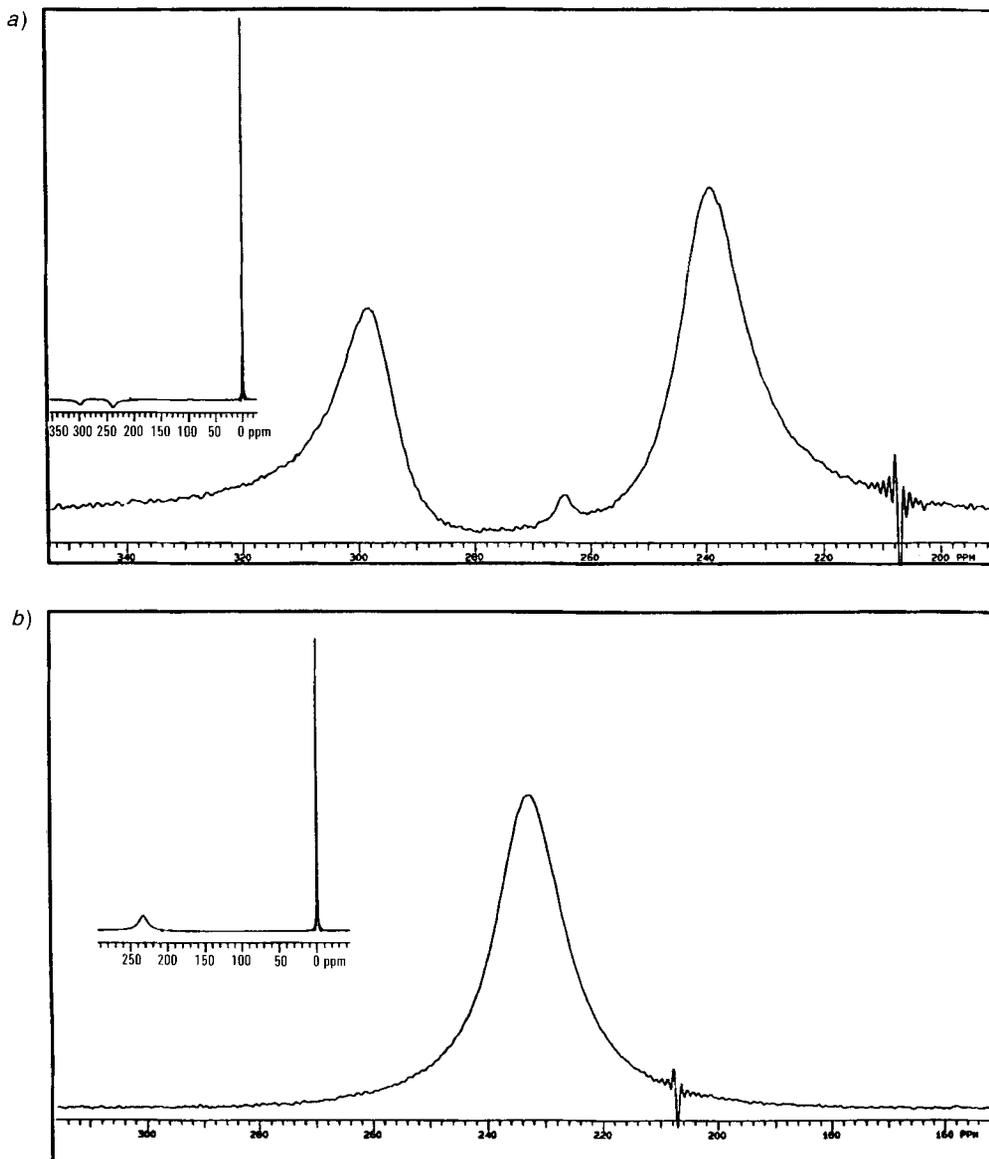


Figure. ^{17}O -NMR Spectrum of a) **12** and b) **14**. In 0.1M sodium phosphate, pH 7.0 at 45°; external standard 1,4-dioxane (0 ppm).

the residual benzoyl groups, only 31% of uridine was isolated, together with 14% of *O*⁴-methyluridine (**u-10**), and 31% of another compound that was identified as DBU substitution product **u-11**, suggests a more complicated mechanism than initially expected (*Scheme 2*).

Apparently, the prolonged reaction time allowed for a nucleophilic attack of unreacted starting material by freshly formed, deprotonated 2',3',5'-tri-*O*-benzoyluridine resulting in considerable amounts of 'dimeric' compound **u-9** (TLC). During the subsequent *in situ* methanolysis, all compounds debenzoylated. TLC confirmed the formation of uridine and presumably debenzoylated 'dimer'¹⁾. Part of **u-9** underwent a substitution 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[¹⁷O]H solution in THF for the hydrolysis of **8a/b**⁵⁾. The melting range and the spectroscopic data of **12** (¹H-, ¹³C-, ¹⁵N-, and ¹⁷O-NMR and FAB-MS (pos.)) confirmed its purity and structure (¹⁵N-NMR: 125.0 (N(1)) and 138.4 ppm (N(3)), *J*(N,N)'s not resolved). The ¹⁷O-NMR spectrum of **12** is depicted in the *Figure*.

(¹⁵N₃)[*O*²⁻¹⁷O]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% ¹⁵NH₄OH solution. Tribenzoylcytidine **13** precipitated almost instantaneously. Workup and chromatographic purification resulted in a recovery of *ca.* 80% of the used ¹⁵NH₃ and 5.6% of unreacted **8a/b** and in a 71% yield of pure **13**. A fraction of **13** was subsequently deprotected in 28% NH₄OH/THF for 2 days at 55° to give pure **14**⁶⁾. The melting range and the spectroscopic data of **14** (¹⁵N- and ¹⁷O-NMR, FAB-MS (pos.)) confirmed its purity and structure (¹⁵N-NMR: 72.2 (N⁴), 131.6 (N(1)), and 181.4 ppm (N(3)), *J*(N,N)'s not resolved). The ¹⁷O-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: (¹⁵N₂)Urea (99 atom-% ¹⁵N) was purchased from *Cambridge Isotope Laboratories*, H₂[^{17/18}O] (3 g; 36 atom-% ¹⁶O, 35 atom-% ¹⁷O, 29 atom-% ¹⁸O, normalized) from *Iso-Yeda Co. Ltd.*, Rehovot, Israel. Less enriched H₂[^{17/18}O] (2.3 ml, 21 atom-% ¹⁷O) was a generous gift from Prof. *Hans Dahn*, Institut de Chimie Organique, Université 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*[®] RP-18 (*Merck*), 15–25 μm (packed as toluene suspension). M.p.: *Kofler* block with digital temp. display; corrected.

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 N⁴ so that it can be further converted into a DNA/RNA-synthesizer-compatible phosphoramidite.

IR Spectra: *Perkin-Elmer-1600-FTIR* spectrometer, *Hewlett-Packard Color Pro* plotter; $\tilde{\nu}$ in cm^{-1} . MS: B = nucleobase fragment. Elemental analysis: the $^{15}\text{N}/^{14}\text{N}$ -correction factor was determined to be insignificant (1.0065 using solid $^{15}\text{NH}_4\text{Cl}$ and $^{14}\text{NH}_4\text{Cl}$).

(^{15}N) *Ammonium* (^{15}N) *Thiocyanate* (**2**). A soln. of $^{15}\text{NH}_4\text{Cl}$ (19.928 g, 366 mmol) in H_2O (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, CSCl_2 (1; 14 ml, 185 mmol; *Aldrich*), then, a separately degassed and cooled (0°) NaOH soln. (29.8 g, 745 mmol) in H_2O (60 ml) were steadily added to the $^{15}\text{NH}_4\text{Cl}$ soln. under an Ar flow and without stirring (CSCl_2 is light and H_2O -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 ($120^\circ/50$ μbar , 3 h) furnished 7.871 g of pure, slightly yellowish **2** (201 mVal ^{15}N , 55.1% rel. to used $^{15}\text{NH}_4\text{Cl}$; 2nd round: 2.509 g (53.4%); 3rd round: 0.592 g (36.2%)). Total yield after 4 rounds: 10.972 g of **2** (281 mVal ^{15}N , 81.6% rel. to consumed $^{15}\text{NH}_4\text{Cl}$). M.p. $140\text{--}150^\circ$. IR (KBr): 3450s (br. sh), 3123s (br.), 2039s, 1624w (br.), 1394s. EI-MS (70 eV): 60 (100, [$^{15}\text{N}_2$] $M - ^{15}\text{NH}_3$), 32 (49, S^+), 18 (29, $^{15}\text{NH}_3$), 17 (23, $^{15}\text{NH}_2$). Anal. calc. for $\text{CH}_4^{15}\text{N}_2\text{S}$ (78.12): C 15.38, H 5.16, N 38.42; found: C 15.90, H 6.40, N 37.90.

Regeneration of Unreacted $^{15}\text{NH}_4\text{Cl}$. Both residual precipitates from workup and resublimation were combined, quartered, and dissolved in H_2O (4×70 ml). Each soln. was placed into a *Parnas-Wagner* distillation apparatus. A 50% aq. NaOH soln. (4×10 ml) was added and the mixture water-vapor distilled. $^{15}\text{NH}_3$ was captured in 6N HCl. The $^{15}\text{NH}_4\text{Cl}$ solns. were repeatedly evaporated, redissolved in H_2O , and reevaporated until the pH was above 5. The combined, HCl-free $^{15}\text{NH}_4\text{Cl}$ soln. was lyophilized overnight: 6.56 g (120 mmol, 32.9%) of $^{15}\text{NH}_4\text{Cl}$ (2nd round 2.282 g (34.8%), 3rd round 1.188 g (52.1%)).

($^{15}\text{N}_2$) *Thiourea* (**3**). Solid **2** (10.582 g, 135 mmol) was kept in a sealed vessel at 160° . After 2 h, the reddish liquid was slowly cooled down to 110° , then rapidly to r.t. The brown-green solid was dissolved in H_2O (10 ml), activated charcoal (210 mg) added, the mixture refluxed for 10 min and filtered, the clear soln. evaporated, and the residue dried overnight under h.v.: 10.227 g (97%) of **2/3** 2:1. The solid was sublimed during 20 min at $110^\circ/5$ μbar . The 1st sublimate was washed down from the cooling finger with MeOH. The 1st residue was thoroughly repowdered and resublimed during 20 min under the same conditions. The 2nd residue consisted of 97–98% pure **3**. The 1st and 2nd sublimates were combined in MeOH, the soln. was transferred to the sealed vessel and evaporated, and the residue dried under h.v. and heated as above. Nine rounds of isomerization and resublimation furnished 7.07 g (66%) of **3** in 95–97% chemical purity. M.p. $176\text{--}179^\circ$. IR (KBr): 3359s/3251s/3157s (br.), 2668w, 1578s, 1481s, 1444m, 1402m, 1080m, 741w, 634w, 512s (br.). EI-MS (70 eV): 78 (100, [$^{15}\text{N}_2$] M), 61 (28, [$^{15}\text{N}_2$] $M - ^{15}\text{NH}_3$), 60 (13, [$^{15}\text{N}_2$] $M - ^{15}\text{NH}_3$). Anal. calc. for $\text{CH}_4^{15}\text{N}_2\text{S}$ (78.12): C 15.38, H 5.16, N 38.42; found: C 15.56, H 5.31, N 37.95.

($^{15}\text{N}_2$) *Cyanamide* (**4**). The whole amount (6.2883 g, 80.5 mmol) of **3** was separated into three 2.096-g portions (26.8 mmol) which were treated separately as follows: Et_2O (30 ml) was added and the suspension vigorously stirred in a NaCl/ice bath. Carefully addition of *HgO flavum* (8.145 g, 37.6 mmol) turned the mixture into a bright orange-red suspension. Subsequent addition of H_2O (363 μl , 20.15 mmol) induced the formation of a black precipitate (HgS). After stirring at -5° for 30 min, a little anh. Na_2SO_4 was added and stirring continued for another 30 min at r.t. The suspension was filtered, the precipitates washed with Et_2O (5×20 ml), and the filtrate carefully evaporated (25 $^\circ/0.4$ bar): 0.887, 0.966, and 0.922 g of a smoke-stained crystalline solid, i.e. 2.775 g (78%) of **4**. M.p. $39\text{--}40^\circ$. IR (KBr): 3360s (br.), 2761w, 2229s, 1626m, 1569m, 700–400m (br.).

($^{15}\text{N}_2$) (^{17}O) *Urea* (**5**). To a soln. of **4** (0.887 g, 20.13 mmol) in H_2 [$^{17/18}\text{O}$] (2.066 ml, 108.7 mmol; 35 atom-% ^{17}O), 12N HCl/ H_2 [$^{17/18}\text{O}$] stock soln. (426 μl), prepared by saturating H_2 [$^{17/18}\text{O}$] (804 μl ; 35 atom-% ^{17}O) with dry HCl gas at 0° , was added under Ar. The soln. was refluxed (120°) under Ar for 25 min, then neutralized at r.t. with an appropriate minimal amount of anh. Na_2CO_3 , and lyophilized to regenerate excess H_2 [$^{17/18}\text{O}$]. The residue was sublimed at 25 μbar giving 91.7 mg (10.3%) of pure unreacted **4** (10–20 min, $50\text{--}75^\circ$) and 0.8475 g (74.2%) of **5** (110– 120° , 2.5 h). The 2nd conversion starting from 1.0414 g of **4** yielded 1.0465 g (81.6%) of **5** and 145.7 mg (14%) of unreacted **4**, the 3rd conversion starting from 1.0468 g of **4** yielded 0.8880 g (78%) of **5** and 0.2516 g (24%) of unreacted **4**, and the 4th conversion gave 0.3131 g (87%) of **5** and no **4**: 3.094 g (75%) of **5**. M.p. $127\text{--}129.5^\circ$. IR (KBr): 3430 (br.), 3340 (br.), 1669s, 1615s, 1458s, 1139m, 800–400 (br.). EI-MS (70 eV): 64 (1st 28.7, 2nd 30.6, 3rd 42.6, 4th 32.2, [^{18}O] M), 63 (1st 34.9, 2nd 36.9, 3rd 52.4, 4th 40.9, [^{17}O] M), 62 (1st 31.9, 2nd 36.7, 3rd 60.4, 4th 51.8, [^{16}O] M), 47 (1st 29.3, 2nd 32.9, 3rd 45.4, 4th 35.5 [^{18}O] $M - ^{15}\text{NH}_2$), 46 (1st 39.2, 2nd 44.1, 3rd 59.2, 4th

48.8, $[(^{17}\text{O})M - ^{15}\text{NH}_2]^+ + [(^{18}\text{O})M - ^{15}\text{NH}_3]^+$, 45 (1st 45.9, 2nd 53.5, 3rd 86.5, 4th 77.3, $[(^{16}\text{O})M - ^{15}\text{NH}_2]^+ + [(^{17}\text{O})M - ^{15}\text{NH}_3]^+$), 44 (1st 9.3, 2nd 11.3, 3rd 17.8, 4th 15.1, $[(^{16}\text{O})M - ^{15}\text{NH}_3]^+$), 18 (100, $^{15}\text{NH}_3$), 17 (19, $^{15}\text{NH}_2$).

$(^{15}\text{N}_2)/\text{O}^{2.17/18}\text{O}/\text{Uracil}$ (**6**). A mechanically stirred mixture of **5** (2.895 g, 45.97 mmol), polyphosphoric acid (80 g) and freshly distilled propynoic acid (3.1 ml, 3.53 g, 50.4 mmol) was heated to 85°. 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 H₂O (160 ml). The resulting light-yellow soln. was stirred for 1 h at r.t. and then cooled down to -10° to crystallize crude **6**. The mixture was neutralized with 6N NaOH and the suspension cooled and filtered. The turbid orange mother liquor was concentrated to ½ of its volume to crystallize a 2nd portion of crude **6**. The combined portions were recrystallized in hot H₂O: 3.08 g (58%) of TLC-pure yellowish solid (TLC (AcOEt/MeOH/H₂O) 4:1:0.2; R_f 0.5). The 2nd mother liquor was evaporated and lyophilized and the residue sublimed (150–200° 5 μbar, +10°/h) 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. ¹H-NMR (300 MHz, CD₃SOCD₃, SiMe₄): 5.46 (*dt*, ³J(H-C(6),H-C(5)) = 7.5, ³J(N(1),H-C(5)) = ³J(N(3),H-C(5)) = 7.5, H-C(5)); 7.39 (*dt*, ³J(H-C(5),H-C(6)) = 7.5, ²J(N(1),H-C(6)) = ⁴J(N(3),H-C(6)) = 7.5, H-C(6)); 10.9, 11.2 (*br.*, H-N(3), H-N(1)). EI-MS (70 eV): 116 (48.2, $[(^{18}\text{O}_1,^{16}\text{O}_1)M]^+$), 115 (58.4, $[(^{17}\text{O}_1,^{16}\text{O}_1)M]^+$), 114 (61.7, $[(^{16}\text{O}_2)M]^+$). Anal. calc. for C₄H₄¹⁵N₂¹⁶O₂/C₄H₄¹⁵N₂¹⁶O¹⁷O/C₄H₄¹⁵N₂¹⁶O¹⁸O 36:35:29 (114.95): C 41.79, H 3.51, N 26.11; found: C 41.43, H 3.51, N 24.47.

2',3',5'-Tri-O-benzoyl(¹⁵N₂)/*O*^{2.17/18}*O*/uridine (**7**) was synthesized according to [14a] using equimolar amounts of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose and SnCl₄ in CH₂ClCH₂Cl at r.t. Pure **7** was obtained after CC (SiO₂, 2% MeOH/CH₂Cl₂). ¹H-NMR (300 MHz, CDCl₃, SiMe₄): 4.68 (*dd*, ³J = 4, ²J = 12, H_a-C(5')); 4.71 (*td*, ³J(H_a-C(5'),H-C(4')) = ³J(H-C(3'),H-C(4')) = 4, ³J(H_b-C(5'),H-C(4')) = 2.5, H-C(4')); 4.84 (*dd*, ³J = 2.5, ²J = 12, H_b-C(5')); 5.61 (*m*, ³J(H-C(6),H-C(5)) = 8, ³J(N(3),H-C(5)) = 2, H-C(5)); 5.76 (*ddd*, ³J(H-C(1'),H-C(2')) = 5.6, ³J(H-C(3'),H-C(2')) = 4.5, ³J(N(1),H-C(2')) = 1.5, H-C(2')); 5.89 (*dd*, ³J(H-C(2'),H-C(3')) = 4.5, ³J(H-C(4'),H-C(3')) = 4, H-C(3')); 6.31 (*d*, ³J(H-C(2'),H-C(1')) = 5.6, H-C(1')); 7.41 (*dd*, ³J(H-C(5),H-C(6)) = 8, ³J(N,H-C(6)) = 2, H-C(6)); 7.34–7.63, 7.93–7.99, 8.09–8.11 (3*m*, 3 Ph); 8.68 (*dd*, ¹J(N(3),H) = 91, ³J(N(1),H) = 2, H-N(3)). ¹³C-NMR (75 MHz, CDCl₃, SiMe₄): 63.70 (C(5')); 71.14 (C(3')); 73.72 (C(2')); 80.51 (C(4')); 88.09 (*d*, ¹J(N(1),C(1')) = 13.8, C(1')); 103.40 (*d*, ³J(N(1),C(5)) = 7, C(5)); 128–130 (C_{ar}, C_m, C_p); 133.66, 133.76, 133.82 (C_{ipso}); 139.56 (*d*, ¹J(N(1),C(6)) = 12.6 C(6)); 149.93 (*t*, ¹J(N(3),C(2)) = ¹J(N(1),C(2)) ≈ 18, C(2)); 162.50 (*d*, ¹J(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''(and 4'')-yl)-2',3',5'-tri-O-benzoyl(¹⁵N₂)/*O*^{2.17/18}*O*/uridine (**8a/b**). To a vigorously stirred suspension of 1*H*-1,2,4-triazole (10.698 g, 154.68 mmol) in dry MeCN (140 ml), POCl₃ (3.48 ml, 38.16 mmol) was added at -10° during 2–3 min, followed by Et₃N (24.8 ml, 177.37 mmol) during 5 min. After 1 h at -5 to 0°, a soln. of **7** (10.31 mmol) in dry MeCN (100 ml) was added and the mixture stirred at r.t. overnight. The orange suspension was rapidly filtered through SiO₂ (200 g; *h* 8 cm, Ø 9 cm; MeCN), the filtrate evaporated, and the mixture submitted to FC (SiO₂ (250 g), MeCN; R_f 0.6): 5.92 g (94%) of **8a/b**. Yellow solid. M.p. 180–183°. ¹H-NMR (300 MHz, CDCl₃, SiMe₄): 4.75 (*dd*, ³J = 4, ²J = 12, H_a-C(5')); 4.85 (*m*, H-C(4')); 4.90 (*dd*, ³J = 2.5, ²J = 12, H_b-C(5')); 5.86 (*td*, ³J(H-C(1'),H-C(2')) = ³J(H-C(3'),H-C(2')) = 4.5, ³J(N(1),H-C(2')) = 1.5, H-C(2')); 5.93 (*t*, ³J(H-C(2'),H-C(3')) = ³J(H-C(4'),H-C(3')) = 5.0, H-C(3')); 6.48 (*d*, ³J(H-C(2'),H-C(1')) = 4.6, H-C(1')); 6.96 (*dt*, ³J(H-C(6),H-C(5)) = 7.4, ³J(N(3),H-C(5)) = ³J(N(1),H-C(5)) = 7.4, H-C(5)); 7.27–7.64, 7.95–8.09, 8.11–8.12 (3*m*, H-C(6), 3 Ph), 8.18, 8.21 (2*s*, H-C(3'), H-C(5')) of **8a**); 9.24 (*s*, H-C(3'), H-C(5')) of **8b**).

*O*⁶-Methyluridine (**u-10**), N⁶-[3-(Hexahydro-2-oxo-1*H*-azepin-1-yl)propyl]cytidine (**u-11**) and Uridine (**u-12**). A soln. of **u-8a/b** (1.377 g, 2.27 mmol), H₂O (163.3 μl, 9.07 mmol), and DBU (1.35 ml, 9.07 mmol) in abs. THF (14 ml) was stirred in a sealed flask under Ar at r.t. After 19 h, TLC showed residual starting material, so more H₂O (41 μl, 2.28 mmol) and DBU (370 μl, 2.28 mmol) were added. After a total reaction time of 25 h, THF was evaporated, CHCl₃ added, and the soln. extracted with aq. NaHCO₃ soln. (2 ×) and H₂O (5 ×), dried (Na₂SO₄), and evaporated. The dark orange foam was dissolved in abs. MeOH and co-evaporated 5 × 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 × 8 (20–50 mesh, H⁺ form), filtered, and evaporated. The crude solid was dissolved in H₂O and the aq. soln. extracted with CHCl₃ and lyophilized: 581 mg. Purification by FC (SiO₂ (30 g), AcOEt/MeOH/H₂O 4:1:0.2) furnished 253 mg of a 2:1 mixture (¹H-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: $^1\text{H-NMR}$ (300 MHz, CD_3SOCD_3 , SiMe_4): 3.56 (*ddd*, $^3J(\text{OH}, \text{CH}_2(5')) = 5.0$, $^2J = 12$, $^3J = 3.3$, $\text{H}_a\text{-C}(5')$, **u-12**, **u-10**); 3.63 (*ddd*, $^3J(\text{OH}, \text{CH}_2(5')) = 5.0$, $^2J = 12$, $^3J = 3.3$, $\text{H}_b\text{-C}(5')$, **u-12**, **u-10**); 3.83 (*s*, $\text{MeO-C}(4)$, **u-10**); 3.85 (*q*, $^3J = 3.5$, $\text{H-C}(4')$, **u-12**, **u-10**); 3.96 (*br.*, $\text{H-C}(3')$, **u-12**, **u-10**); 4.02 (*m*, $\text{H-C}(2')$, **u-12**, **u-10**); 5.02 (*d*, $^3J(\text{H-C}(3'), \text{OH-C}(3')) = 4.3$, $\text{OH-C}(3')$, **u-10**); 5.09 (*m*, $\text{OH-C}(5')$, $\text{OH-C}(3')$, **u-12**); 5.15 (*t*, $^3J(\text{H}_b\text{-C}(5'), \text{OH-C}(5')) = 5.0$, $\text{OH-C}(5')$, **u-10**); 5.37 (*d*, $^3J(\text{H-C}(2'), \text{OH-C}(2')) = 5.4$, $\text{OH-C}(2')$, **u-12**); 5.45 (*d*, $^3J(\text{H-C}(2'), \text{OH-C}(2')) = 4$, $\text{OH-C}(2')$, **u-10**); 5.64 (*d*, $^3J(\text{H-C}(6), \text{H-C}(5)) = 8.2$, $\text{H-C}(5)$, **u-12**); 5.78 (*d*, $^3J(\text{H-C}(2'), \text{H-C}(1')) = 5.4$, $\text{H-C}(1')$, **u-12**, **u-10**); 6.05 (*d*, $^3J(\text{H-C}(6), \text{H-C}(5)) = 7.4$, $\text{H-C}(5)$, **u-10**); 7.89 (*d*, $^3J(\text{H-C}(5), \text{H-C}(6)) = 8.2$, $\text{H-C}(6)$, **u-12**); 8.32 (*d*, $^3J(\text{H-C}(5), \text{H-C}(6)) = 7.4$, $\text{H-C}(6)$, **u-10**); 11.29 (*br. s*; $\text{H-N}(3)$, **u-12**). $^{13}\text{C-NMR}$ (75 MHz, CD_3SOCD_3 , SiMe_4): 54.52 ($\text{MeO-C}(4)$, **u-10**); 60.55 ($\text{C}(5')$, **u-10**); 61.33 ($\text{C}(5')$, **u-12**); 69.37 ($\text{C}(3')$, **u-10**); 70.35 ($\text{C}(3')$, **u-12**); 73.97 ($\text{C}(2')$, **u-12**); 74.90 ($\text{C}(2')$, **u-10**); 84.71 ($\text{C}(4')$, **u-10**); 85.33 ($\text{C}(4')$, **u-12**); 88.25 ($\text{C}(1')$, **u-12**); 90.36 ($\text{C}(1')$, **u-10**); 95.46 ($\text{C}(5)$, **u-10**); 102.37 ($\text{C}(5)$, **u-12**); 141.45 ($\text{C}(6)$, **u-12**); 144.83 ($\text{C}(6)$, **u-10**); 151.27 ($\text{C}(2)$, **u-12**); 155.79 ($\text{C}(2)$, **u-10**); 163.92 ($\text{C}(4)$, **u-12**); 171.80 ($\text{C}(4)$, **u-10**). FAB-MS (*pos.*, nitrobenzyl alcohol): 259 (51.7 [$M + \text{H}$] $^+$, **u-10**), 245 (31.9, [$M + \text{H}$] $^+$, **u-12**), 127 (100, [$B + 2\text{H}$] $^+$, **u-10**), 113 (41.8, [$B + 2\text{H}$] $^+$, **u-12**), 89 (10.6), 77 (9.8).

u-11: $^1\text{H-NMR}$ (300 MHz, CD_3SOCD_3 , SiMe_4): 1.62 (*m*, 8 H, $4 \times \text{CH}_2$); 2.42 (*m*, 2 H, CH_2); 3.23 (*t*, $^3J = 7$, 2 H, CH_2); 3.33 (*m*, 4 H, CH_2); 3.56 (*d*, $^2J(\text{H}_b\text{-C}(5'), \text{H}_a\text{-C}(5')) = 12$, $\text{H}_a\text{-C}(5')$); 3.66 (*d*, $^2J(\text{H}_a\text{-C}(5'), \text{H}_b\text{-C}(5')) = 12$, $\text{H}_b\text{-C}(5')$); 3.82 (*br.*, $\text{H-C}(4')$); 3.94 (*br.*, $\text{H-C}(3')$, $\text{H-C}(2')$); 5.00 (*br. s*, $\text{OH-C}(3')$, $\text{OH-C}(5')$); 5.28 (*br. s*, $\text{OH-C}(2')$); 5.72 (*d*, $^3J(\text{H-C}(6), \text{H-C}(5)) = 7.4$, $\text{H-C}(5)$); 5.76 (*d*, $^3J(\text{H-C}(2'), \text{H-C}(1')) = 3.2$, $\text{H-C}(1')$); 7.65 (*t*, $^3J = 5.3$, $\text{NH-C}(4)$); 7.78 (*d*, $^3J(\text{H-C}(5), \text{H-C}(6)) = 7.4$, $\text{H-C}(6)$). $^{13}\text{C-NMR}$ (75 MHz, CD_3SOCD_3 , SiMe_4): 23.37 (CH_2); 27.70 (CH_2); 28.68 (CH_2); 29.55 (CH_2); 36.84 (CH_2); 37.81 (CH_2); 45.55 (CH_2); 48.99 (CH_2); 60.93 ($\text{C}(5')$); 69.71 ($\text{C}(3')$); 74.18 ($\text{C}(2')$); 84.35 ($\text{C}(4')$); 89.49 ($\text{C}(1')$); 95.05 ($\text{C}(5)$); 140.62 ($\text{C}(6)$); 155.87 ($\text{C}(2)$); 163.57 ($\text{C}(4)$); 175.27 ($\text{C}=\text{O}$). FAB-MS (*pos.*, nitrobenzyl alcohol): 397 (100, [$M + \text{H}$] $^+$), 265 (73.8, [$M - \text{ribosyl} + \text{H}$] $^+$), 152 (17.1, [$M - 245 + \text{H}$] $^+$), 138 (11.0), 125 (8.20), 112 (5.2), 98 (6.6).

($^{15}\text{N}_2$)/ O_2 , $^{17/18}\text{O}_2$]Uridine (**12**). A $\text{Na}^{17/18}\text{O}$]H soln. containing Na (262 mg, 11.4 mmol) in H_2 [$^{17/18}\text{O}$] (0.9 ml, 35 atom-% ^{17}O) was prepared as described for the synthesis of **10** in [6]. The soln. was mixed with THF (3 ml), and a soln. of **8a/b** (2.3 g, 3.77 mmol) in THF (10 ml + 2 \times 5 ml rinse) was added at r.t. The flask was sealed and the yellow suspension stirred at 50° for 68 h. TLC indicated that all starting material had reacted but debenzoylation was incomplete. The mixture was neutralized with Dowex 50 $W \times 8$ (20–50 mesh, H^+ form), lyophilized, and suspended in CHCl_3 , the resin was filtered off, and the solvent evaporated. THF (10 ml) was added, followed by portionwise addition of H_2 [$^{17/18}\text{O}$] (2.3 ml, 21 atom-% ^{17}O) containing Na (370 mg; prepared as above) within 24 h. The mixture was stirred in a sealed flask at 70°. After TLC (AcOEt/MeOH/ H_2O 4:1:0.3) indicated complete conversion to **12** (R_f 0.5), the suspension was neutralized as above and the clear resin-containing soln. filtered and extracted with $\text{CHCl}_3/\text{H}_2\text{O}$. The aq. phase was evaporated and lyophilized to give 839 mg of crude **12**. Reversed-phase MPLC (eluant $A = \text{H}_2\text{O}$, eluant $B = 30\%$ MeCN/ H_2O ; gradient: 1 min A , 120 min 0–50% B , 10 min 50% B , 10 min 50–100% B , 60 min 100% B , 10 min 100–0% B) furnished 375 mg (40%) of **12**. Colorless solid. M.p. 163–165°. $^1\text{H-NMR}$ (300 MHz, CD_3SOCD_3 , SiMe_4): 3.55 (*dd*, $^3J = 2.8$, $^2J = 12$, $\text{H}_a\text{-C}(5')$); 3.63 (*dd*, $^3J = 2.8$, $^2J = 12$, $\text{H}_b\text{-C}(5')$); 3.84 (*m*, $\text{H-C}(4')$); 3.97 (*m*, $\text{H-C}(3')$); 4.03 (*t*, $^3J = 5$, $\text{H-C}(2')$); 5.1 (*br. s*, $\text{OH-C}(5')$, $\text{OH-C}(3')$); 5.37 (*br. s*, $\text{OH-C}(2')$); 5.63 (*m*, $\text{H-C}(5)$); 5.78 (*d*, $^3J = 5$, $\text{H-C}(1')$); 7.88 (*dd*, $^3J(\text{H-C}(5), \text{H-C}(6)) = 8$, $^3J(\text{N}(1), \text{H-C}(6)) = 2$, $\text{H-C}(6)$); *ca.* 11.3 (*br. s*, $\text{H-N}(3)$). $^{13}\text{C-NMR}$ (101 MHz, CD_3SOCD_3 , SiMe_4): 60.81 ($\text{C}(5')$); 69.85 ($\text{C}(3')$); 73.47 ($\text{C}(2')$); 84.82 ($\text{C}(4')$); 87.69 ($^1J(\text{N}(1), \text{C}(1')) = 12.2$, $\text{C}(1')$); 101.80 (*d*, $^3J(\text{N}, \text{C}(5)) = 6.4$, $\text{C}(5)$); 140.78 (*d*, $^1J(\text{N}(1), \text{C}(6)) = 12.2$, $\text{C}(6)$); 150.83 (*t*, $^1J(\text{N}(1), \text{C}(2)) = ^1J(\text{N}(3), \text{C}(2)) = 17.5$, $\text{C}(2)$); 163.17 (*d*, $^1J(\text{N}(3), \text{C}(4)) = 9.3$, $\text{C}(4)$). $^{15}\text{N-NMR}$ (41 MHz, ^1H -broadband-decoupled, 0.1M aq. sodium phosphate buffer, pH 7.0, 5% (*v/v*) D_2O , 25°, internal $^{15}\text{NH}_4\text{Cl}$): 124.98 (*br. s*, $\text{N}(1)$); 138.42 (*br. s*, $\text{N}(3)$). $^{17}\text{O-NMR}$ (54 MHz, 0.1M aq. sodium phosphate buffer, pH 7.0, 45°, external 1,4-dioxane (*neat*)): 238.9 (*br. s*, $w_{1/2} \approx 690^\circ$, $\text{O-C}(2)$); 297.9 (*br. s*, $w_{1/2} \approx 740$, $\text{O-C}(4)$). FAB-MS (*pos.*, glycerine/ H_2O): 252 (4.0, [$^{18}\text{O}_2$] $M + 2\text{H}$] $^+$), 251 (20.5, [$^{18}\text{O}_1, ^{17}\text{O}_1$] $M + 2\text{H}$] $^+$ + [$^{18}\text{O}_2$] $M + \text{H}$] $^+$), 250 (43.4, [$^{17}\text{O}_2$] $M + 2\text{H}$] $^+$ + [$^{18}\text{O}_1, ^{16}\text{O}_1$] $M + 2\text{H}$] $^+$ + [$^{18}\text{O}_1, ^{17}\text{O}_1$] $M + \text{H}$] $^+$), 249 (91.2, [$^{17}\text{O}_1, ^{16}\text{O}_1$] $M + 2\text{H}$] $^+$ + [$^{17}\text{O}_2$] $M + \text{H}$] $^+$ + [$^{18}\text{O}_1, ^{16}\text{O}_1$] $M + \text{H}$] $^+$), 248 (85.7, [$^{16}\text{O}_2$] $M + 2\text{H}$] $^+$ + [$^{17}\text{O}_1, ^{16}\text{O}_1$] $M + \text{H}$] $^+$), 247 (69.9, [$^{16}\text{O}_2$] $M + \text{H}$] $^+$), 133 (43.9), 120 (0.7, [$^{18}\text{O}_2$] $B + 2\text{H}$] $^+$), 119 (16.3, [$^{18}\text{O}_1, ^{17}\text{O}_1$] $B + 2\text{H}$] $^+$ + [$^{18}\text{O}_2$] $B + \text{H}$] $^+$), 118 (35.6, [$^{17}\text{O}_2$] $B + 2\text{H}$] $^+$ + [$^{18}\text{O}_1, ^{16}\text{O}_1$] $B + 2\text{H}$] $^+$ + [$^{18}\text{O}_1, ^{17}\text{O}_1$] $B + \text{H}$] $^+$), 117 (100.0, [$^{17}\text{O}_1, ^{16}\text{O}_1$] $B + 2\text{H}$] $^+$ + [$^{17}\text{O}_2$] $B + \text{H}$] $^+$ + [$^{18}\text{O}_1, ^{16}\text{O}_1$] $B + \text{H}$] $^+$), 116 (95.2, [$^{16}\text{O}_2$] $B + 2\text{H}$] $^+$ + [$^{17}\text{O}_1, ^{16}\text{O}_1$] $B + \text{H}$] $^+$), 115 (90.1, [$^{16}\text{O}_2$] $B + \text{H}$] $^+$), 97 (16.8), 73 (23.2).

⁷) δ (*ext. neat* $\text{Me}^{15}\text{NO}_2$) = 360.7 ppm + δ (*int. aq.* $^{15}\text{NH}_4\text{Cl}$).

⁸) Signal width [Hz] at half the signal intensity.

2',3',5'-Tri-O-benzoyl($^{15}\text{N}_3$)[O $^{2-17/18}\text{O}$]cytidine (**13**). A soln. 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% $^{15}\text{NH}_4\text{OH}$ soln. (ca. 12 ml) that was prepared by adding 30 ml of an aq. soln. containing $^{15}\text{NH}_4\text{Cl}$ (10 g, 183.5 mmol) to a conc. soln. containing NaOH (97 g) and H_2O (97 ml), heating it with an electric fan, and capturing the $^{15}\text{NH}_3$ gas through a distillation bridge into a cooled flask (liquid N_2) containing H_2O (8.16 g). Immediately after addition of **8a/b**, an orange precipitate formed. After stirring the suspension for 5 h at r.t., TLC (AcOEt/MeOH/ H_2O 4:1.0:2) showed almost complete conversion to **13** (R_f 0.7) with no sign of **14** (R_f 0.18). The liquid was pipetted into a separate flask which, as described above, was connected to a cooled flask containing H_2O (6.4 ml), and was heated to regenerate 2.56 g (ca. 142 mmol, 80%) of $^{15}\text{NH}_3$ (as a ca. 28% $^{15}\text{NH}_4\text{OH}$ soln.). The residual solid was dissolved in CHCl_3 , the soln. evaporated, and the residue dried under h.v. (4 h). The crude product (2.79 g) was purified by FC (SiO_2 (135 g), 0–30% MeOH/MeCN) to give 0.2 g (5.6%) of unreacted **8a/b** and 2.21 g (71%) of **13**. $^1\text{H-NMR}$ (300 MHz, CDCl_3 , SiMe_4): 4.65 (dd, $^3J = 4.5$, $^2J = 12$, $\text{H}_a\text{-C}(5')$); 4.70 (dd, $^3J = 2.5$, $^2J = 12$, $\text{H}_b\text{-C}(5')$); 4.79 (m, $\text{H-C}(4')$); 5.85–5.94 (m, $\text{H-C}(2')$, $\text{H-C}(5)$); 5.97 (t, $^3J = 6$, $\text{H-C}(3')$); 6.15 (d, $^3J(\text{H-C}(2'), \text{H-C}(1')) = 3.8$, $\text{H-C}(1')$); 6.70 (br. d, $^1J(\text{N}^4, \text{H}) \approx 90$, $\text{NH}_2\text{-C}(4)$); 7.31–7.40, 7.42–7.56 (2m, 9 arom. H, $\text{H-C}(6)$); 7.90–7.95, 8.03–8.07, 8.16–8.17 (3m, 6 arom. H). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3 , SiMe_4): 63.71 (C(5')); 71.04 (C(3')); 74.45 (C(2')); 79.61 (C(4')); 90.92 (d, $^1J(\text{N}(1), \text{C}(1')) = 13.2$, C(1')); 96.15 (C(5)); 128–130 ($\text{C}_o, \text{C}_m, \text{C}_p$); 133.35, 133.56 (C_{ipso}); 141.44 (d, $^1J(\text{N}(1), \text{C}(6)) = 11.9$, C(6)); ca. 155.6 (t, C(2)); ca. 162 (t, C(4)); 165.27, 165.40, 166.20 (PhCO). FAB-MS (pos., nitrobenzyl alcohol): 562 (1.1, $[(^{18}\text{O}_1)\text{M} + 2\text{H}]^+$), 561 (4.0, $[(^{17}\text{O}_1)\text{M} + 2\text{H}]^+ + [(^{18}\text{O}_1)\text{M} + \text{H}]^+$), 560 (4.8, $[(^{16}\text{O}_1)\text{M} + 2\text{H}]^+ + [^{17}\text{O}_1)\text{M} + \text{H}]^+$), 559 (4.3, $[(^{16}\text{O}_1)\text{M} + \text{H}]^+$), 446 (9.8, $[\text{M} - \text{B} + \text{H}]^+$), 445 (32.5, $[\text{M} - \text{B}]^+$), 201 (12.6), 117 (4.2, $[(^{18}\text{O}_1)\text{B} + 2\text{H}]^+$), 116 (5.1, $[(^{17}\text{O}_1)\text{B} + 2\text{H}]^+$), 115 (6.9, $[(^{16}\text{O}_1)\text{B} + 2\text{H}]^+$), 105 (100.0, PhCO $^+$).

($^{15}\text{N}_3$)[O $^{2-17/18}\text{O}$]cytidine (**14**). A mixture of **13** (178 mg, 0.32 mmol) and 0.236N NaOMe/MeOH (3 ml) was stirred for 1 h at r.t. The soln. was neutralized with a minimal amount of Dowex 50W \times 8 (20–50 mesh, H^+ form), filtered, and evaporated to give 62.8 mg (80%) of colorless **14**. M.p. 209–213° (dec.). $^{15}\text{N-NMR}$ (41 MHz, ^1H -broadband-decoupled, 0.1M aq. sodium phosphate buffer, pH 7.0, 5% (v/v) D_2O , 25°, internal $^{15}\text{NH}_4\text{Cl}$): 72.20 (br. s, N–C(4)); 131.61 (s, N(1)); 181.37 (br. s, N(3)). $^{17}\text{O-NMR}$ (54 MHz, 0.1M aq. sodium phosphate buffer, pH 7.0, 45°, external 1,4-dioxane (neat)): 232.3 (br. s, $w_\nu \approx 760^8$), O–C(2). FAB-MS (pos., glycerine/ H_2O): 249 (34.0, $[(^{18}\text{O}_1)\text{M} + \text{H}]^+$), 248 (40.9, $[(^{17}\text{O}_1)\text{M} + \text{H}]^+$), 247 (44.8, $[(^{16}\text{O}_1)\text{M} + \text{H}]^+$), 117 (63.5, $[(^{18}\text{O}_1)\text{B} + 2\text{H}]^+$), 116 (79.7, $[(^{17}\text{O}_1)\text{B} + 2\text{H}]^+$), 115 (100.0, $[(^{16}\text{O}_1)\text{B} + 2\text{H}]^+$).

REFERENCES

- [1] G. M. Clore, A. M. Gronenborn, *Science* **1991**, 252, 1390; A. T. Brünger, G. M. Clore, A. M. Gronenborn, R. Saffrich, M. Nilges, *ibid.* **1993**, 261, 328; E. P. Nikonowicz, A. Pardi, *Nature (London)* **1992**, 355, 184; B. T. Farmer, L. Müller, E. P. Nikonowicz, A. Pardi, *J. Am. Chem. Soc.* **1993**, 115, 11040.
- [2] X. Gao, R. A. Jones, *J. Am. Chem. Soc.* **1987**, 109, 3169; B. Goswami, B. L. Gaffney, R. A. Jones, *ibid.* **1993**, 115, 3832.
- [3] P. Strazewski, *Helv. Chim. Acta* **1995**, 78, 1112.
- [4] A. C. Niemann, M. Meyer, T. Engeloch, O. Botta, A. Hädener, P. Strazewski, *Helv. Chim. Acta* **1995**, 78, 421.
- [5] E. P. Nikonowicz, A. Sirt, P. Legault, F. M. Jucker, L. M. Baer, A. Pardi, *Nucleic Acids Res.* **1992**, 20, 4515; R. T. Batey, M. Inada, E. Kujawinski, J. D. Puglisi, J. R. Williamson, *ibid.* **1992**, 20, 4515.
- [6] A. Amantea, M. Walser, U. Séquin, P. Strazewski, *Helv. Chim. Acta* **1995**, 78, 1106.
- [7] B. Rathke, *Ann. Chem. Pharm.* **1873**, 157, 195.
- [8] 'Houben-Weyl, Methoden der organischen Chemie', Georg Thieme Verlag, Stuttgart, 1955, Vol. IX, p. 786–803.
- [9] *Koppers Co.* of Delaware, Pat. (F) 1933, F.P. 730 682 (1932), 1.
- [10] E. Winterstein, A. Küng, *Hoppe-Seyler's Z. Physiol. Chem.* **1909**, 59, 141.
- [11] 'Houben-Weyl, Methoden der organischen Chemie', Georg Thieme Verlag, Stuttgart, 1952, Vol. VIII, p. 96.
- [12] E. D. Korn, in 'Methods in Enzymology', Eds. S. P. Colowick and N. O. Kaplan, Academic Press, New York, 1957, Vol. 4, pp. 623.
- [13] R. L. Lipnick, J. D. Fissekis, *J. Labelled Compd. Radiopharm.* **1980**, 17, 247.
- [14] a) H. Vorbrüggen, K. Krolkiewicz, B. Bennua, *Chem. Ber.* **1981**, 114, 1234; b) H. Vorbrüggen, G. Höfle, *ibid.* **1981**, 114, 1256.
- [15] B. F. L. Li, C. B. Reese, P. F. Swann, *Biochemistry* **1987**, 26, 1086.
- [16] Y. Z. Xu, P. F. Swann, *Nucleic Acids Res.* **1990**, 18, 4061; F. D. Fernandez, Y. Palom, S. Ikuta, E. Pedroso, R. Eritja, *ibid.* **1990**, 18, 5729.
- [17] H. Lammers, P. Cohen-Fernandes, C. L. Habraken, *Tetrahedron* **1994**, 50, 865.