90. Synthesis and ^{15}N- and ^{17}O-NMR Spectra of 5-Methyl($^{15}N_2$)[O^2,O^4-$^{17}O_2$]uridine (= ($^{15}N_2$)[O^2,O^4-$^{17}O_2$]Ribosylthymine)

by **Antonio Amantea, Markus Walser, Urs Séquin,** and **Peter Strazewski***

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

(8.II.95)

The 5-methyl($^{15}N_2$)[O^2,O^4-$^{17}O_2$]uridine (= ($^{15}N_2$)[O^2,O^4-$^{17}O_2$]ribosylthymine; **15**) was synthesized and analyzed by ^{15}N- and ^{17}O-NMR spectroscopy. ($^{15}N_2$)Urea was condensed with 2,3-dibromo-2-methylpropanoyl chloride (**3**) and cyclized to form ($^{15}N_2$)thymine (**5**). After glycosidation, the ^{17}O isotopes were introduced in two separate steps: hydrolytic ring opening of 2,5′-anhydro derivative **9** and hydrolysis of 3-nitro-1*H*-1,2,4-triazole derivative **12** with labelled water in the presence of a strong base. The ^{15}N- and ^{17}O-NMR spectra (*Fig.*) of **15** in phosphate-buffered water serve as references for heteronuclear NMR spectra of labelled RNA fragments.

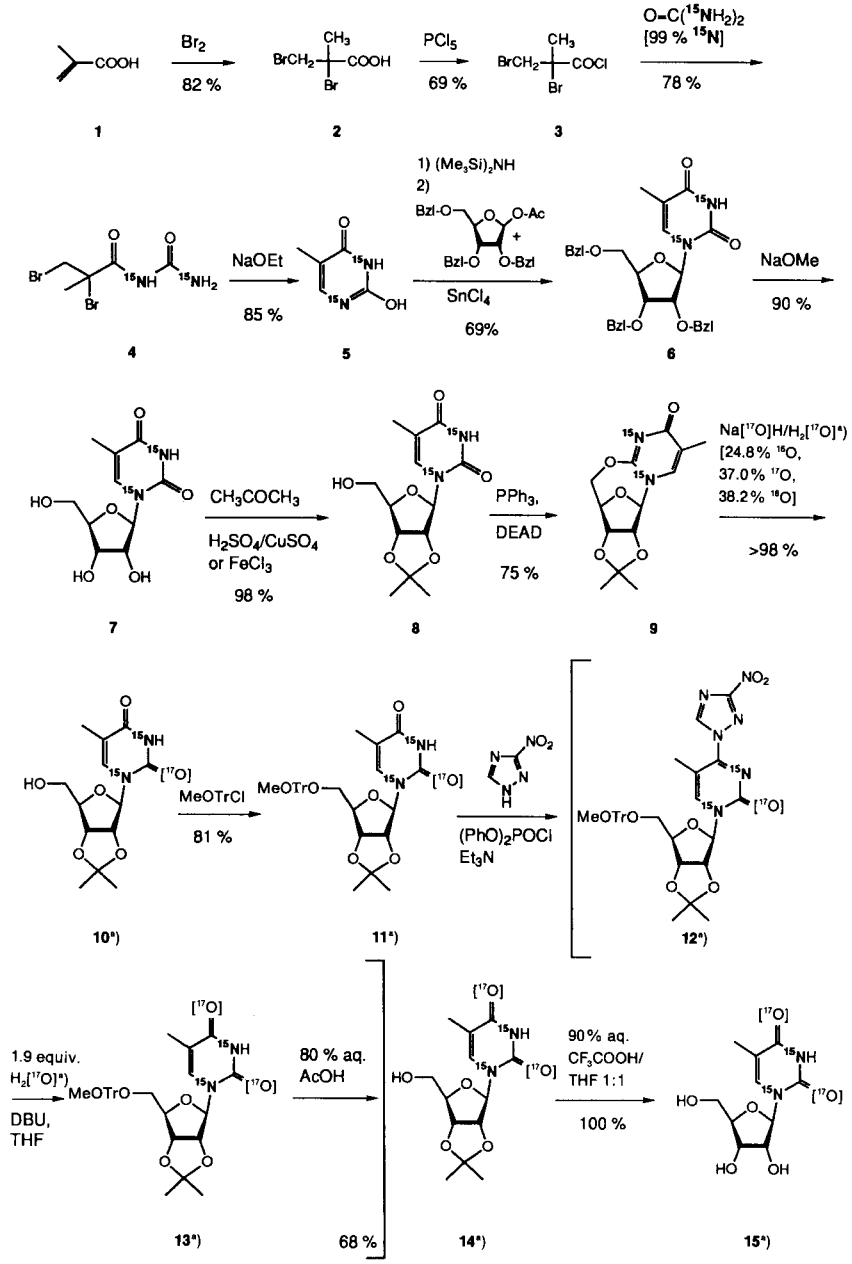
The chemical shifts of both ^{15}N and ^{17}O nuclei are known to be sensitive to the formation of H-bonds. When incorporated into the bases of RNA, these nuclei might serve as a monitoring device for folding and unfolding processes. Doubly labelled 5-methyluridine (= ribosylthymine) was chosen for a first analysis by ^{15}N- and ^{17}O-NMR spectroscopy on the nucleoside level (see also following paper).

The synthesis of 5-methyl($^{15}N_2$)[O^2,O^4-$^{17}O_2$]uridine (= ($^{15}N_2$)[O^2,O^4-$^{17}O_2$]ribosylthymine; **15**) is outlined in the *Scheme*. Thus, 2-methylacrylic acid (= 2-methylprop-2-enoic acid; **1**) is brominated to form 2,3-dibromo-2-methylpropanoic acid (**2**). After transformation into the acyl chloride **3**, condensation with ($^{15}N_2$)urea (99 atom-% ^{15}N) furnishes the *N*-acylurea derivative **4**. ($^{15}N_2$)Thymine (**5**) is obtained through cyclization of **4** under basic conditions [1]. Glycosidation under *Vorbrüggen* conditions [2] (→ **6**) and debenzoylation yields ($^{15}N_2$)nucleoside **7**.

Protection of the 2′- and 3′-OH groups of **7** (→ **8**) and cyclization of the 5′-OH group with one base carbonyl group yields 2,5′-anhydro derivative **9**. The first ^{17}O isotope is introduced at C(2) through the hydrolysis of **9** with a Na[^{17}O]H/H_2[^{17}O] solution giving the doubly labelled nucleoside derivative **10** [3]. Compound **10** is monomethoxytritylated to **11**. The introduction of the second ^{17}O isotope at C(4) is accomplished in three *in situ* steps to avoid loss of material owing to unnecessary purification procedures. The introduction of a leaving group, 3-nitro-1*H*-1,2,4-triazole [4], proceeds efficiently (→ **12**), as judged by TLC and ^1H-NMR. Subsequent hydrolysis with less than 2 equiv. of H_2[^{17}O] yields compound **13**. Detritylation furnishes isopropylidene derivative **14** in 68% overall yield over those steps. Final deprotection with CF_3COOH affords the target compound **15**.

Nucleoside **15** ('rT') is characterized by mass, and ^{17}O- and ^{15}N-NMR spectroscopy (*Fig.*). Comparison of the mass spectra of nucleosides **7**, **10**, and **15** reveals that the isotope-substitution efficiencies are 92% (34 atom-% ^{17}O) for the first and 80% (29.7

Scheme. *Synthesis of 5-Methyl-($^{15}N_2$)[O^2,O^4-$^{17}O_2$]uridine* (**15**) *from 2-Methylacrylic Acid, ($^{15}N_2$)Urea, 1-O-Acetyl-2,3,5-tris-O-benzoyl-β-D-ribofuranose, and H_2[^{17}O]*



Bzl = benzoyl, DEAD = diethyl azodicarboxylate, MeOTr = mono(*p*-methoxy)trityl, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, THF = tetrahydrofuran

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

atom-% ^{17}O) for the second O isotope[1]). The heteronuclear NMR spectra of **15** in phosphate-buffered water (pH 7.0) are depicted in the *Figure* and serve as reference spectra for those of future 'rT'-containing oligoribonucleotides.

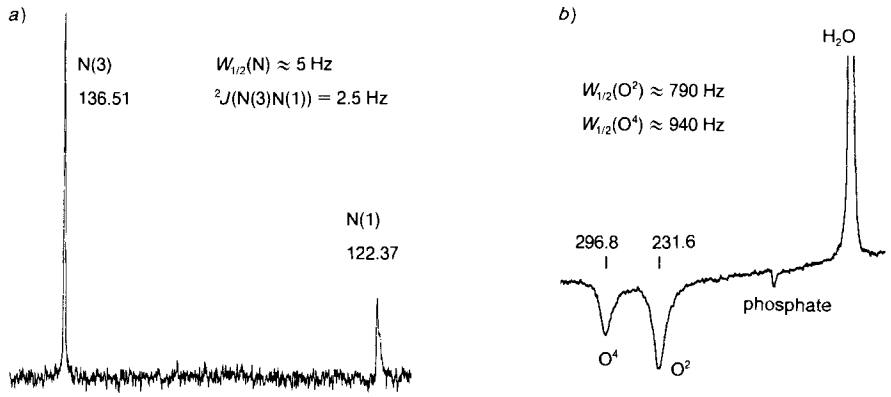


Figure. a) *^{15}N-NMR* (internal standard $^{15}NH_4Cl$ (0 ppm)) *and* b) *^{17}O-NMR Spectrum* (external standard 1,4-dioxane (0 ppm)) *of 5-methyl($^{15}N_2$)[O^2,$O^{4-17}O_2$]uridine* (**15**) *in H_2O* (pH 7.0). For conditions, see *Exper. Part.*

We thank Prof. *Hans Dahn* and Dr. *Peter Péchy*, Institut de Chimie Organique, Université de Lausanne, Lausanne-Dorigny, for introducing us into theoretical and practical aspects of ^{17}O-NMR spectroscopy. The financial support from the *Swiss National Science Foundation* is gratefully acknowledged.

Experimental Part

General. All solvents were distilled or anal. grade. Pyridine was refluxed over CaH_2 (12 h), distilled, and stored over BaO. Fine chemicals were purchased from *Fluka*, unless stated otherwise. ($^{15}N_2$)Urea (99 atom-% ^{15}N) was purchased from *Cambridge Isotope Laboratories*, $H_2[^{17/18}O]$ (3 g, 24.8 atom-% ^{16}O, 37 atom-% ^{17}O, 38.2 atom-% ^{18}O, normalized) from *Iso-Yeda Co. Ltd.*, Rehovot, Israel. Column chromatography (CC) and flash chromatography (FC): silica gel *60*, 63–200 and 40–63 μm, resp. (*Merck*). Thin-layer chromatography (TLC): silica gel *60-F_{254}* (*Merck*); detection under UV light and by dil. methanolic H_2SO_4 soln. followed by 2% (*w/v*) ethanolic naphthoresorcinol soln. and heating. M.p.: *Kofler* block, corrected. B.p.: uncorrected. HPLC: *Shimadzu LC-7A*; high-pressure mixing system, column oven, UV detection at variable wavelength (*Uvikon-722-LC* spectrometer, *Kontron*), digital peak integration (*HP 3380A*, *Hewlett-Packard*). $[\alpha]_D$: *Perkin-Elmer-141* polarimeter; 1-dm cell. IR Spectra: *Perkin-Elmer-781* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Varian-Gemini-300* and *Varian-VXR-400* spectrometers; δ(H), δ(C), δ(N), and δ(O) in ppm rel. to internal or external standards, as indicated for each individual case, scalar coupling constant J in Hz. ^{15}N-NMR: 10-mm (o.d.) sample tube; digital temp. control; spectral width 25 kHz, acquisition time 640 ms, pulse width 90° (21.0 μs), pre-acquisition delay 5.0 s, ^1H-broadband decoupling with WALTZ sequence; double-precision 65536 B-*Fourier* transformation with zero *K* filling, no line broadening. ^{17}O-NMR: 10-mm (o.d.) sample tube; digital temp. control; spectral width 50 kHz, acquisition time 100 ms, pulse width 90° (45.0 μs), no pre-acquisition delay, no ^1H decoupling; no ^2H lock; double-precision 65536 B-*Fourier* transformation with zero *K* filling, line broadening (l.b.) 50–200 Hz; half-intensity signal widths $w_{1/2}$ are given after subtraction of l.b. MS: *VG 70–250*; mass peaks in *m/z* (%); EI (electronic ionization), CI (chemical ionization), or FAB (fast-atom bombardment); B = nucleobase fragment.

2,3-Dibromo-2-methylpropanoic Acid (**2**). A soln. of 1-methylprop-2-enoic acid (**1**; 42.5 ml, 0.5 mol) in $CHCl_3$ (130 ml) was heated to 40–45°. During 45 min, a soln. of Br_2 (27 ml, 0.505 mol) in $CHCl_3$ (50 ml) was added dropwise. The temp. rose to *ca.* 60° and remained without additional heating. After another 2.5 h, the mixture was cooled down and carefully extracted with 5% $NaHCO_3$ soln. (1 × 600 and 3 × 200 ml). The aq. extracts were

[1]) The detailed analysis is described in the thesis of *A. A.*, University of Basel, 1995.

acidified to pH 2 (colourless precipitate) and extracted with CH_2Cl_2 (5 × 200 and 1 × 100 ml). The org. extracts were dried (Na_2SO_4) and evaporated, and the resulting yellowish solid was distilled *in vacuo*: 100.96 g (82%) of **2**. Colourless solid. M.p. 43.6–45.3°. B.p. 99–102°/6 mbar. IR (KBr): 3300–2400 (br.), 1710*s*, 1450, 1380, 1300, 1200, 1045. ^{1}H-NMR (300 MHz, $CDCl_3$, $SiMe_4$): 2.07 (*s*, Me); 3.76 (*d*, ^2J = 10, H_a–C(3)); 4.25 (*d*, ^2J = 10, H_b–C(3)); 11.0 (br. *s*, OH). ^{13}C-NMR (75 MHz, $CDCl_3$, $SiMe_4$): 26.2 (Me); 37.5 (C(3)); 55.0 (C(2)); 175.5 (C(1)). CI-MS (NH_3): 266 (13), 264 (27.5), 262 (13.5, $[M + NH_4]^+$), 184, 182 (10, $[M - HBr]^+$), 138 (21), 136 (22), 104 (81, $[M + NH_4 - 2\,Br]^+$), 66 (99), 58 (51), 56 (48), 39 (100). Anal. calc. for $C_4H_6Br_2O_2$ (248.99): C 19.54, H 2.46, Br 64.99; found: C 19.48, H 2.46, Br 65.17.

2,3-Dibromo-2-methylpropanoyl Chloride (**3**). Neat **2** (6.033 g, 24.53 mmol) was mixed with PCl_5 (4.8 g, 23.05 mmol) and mechanically stirred for 1 h (after 5 min, liquid and clear mixture). Fractionated vacuum distillation (60°/15 mbar, then 110°) gave 4.219 g (69%) of **3**. B.p. 84–85°/25 mbar. IR (NaCl): 3040, 2980, 2935, 1780*s*, 1450, 1420, 1380, 1220, 1050, 980, 870. ^{1}H-NMR (400 MHz, $CDCl_3$, $SiMe_4$): 2.13 (*d*, ^4J = 0.8, Me); 3.78 (*d*, ^2J = 10.5, H_a–C(3)); 4.23 (*dd*, ^2J = 10.5, ^4J = 0.8, H_b–C(3)). ^{13}C-NMR (101 MHz, $CDCl_3$, $SiMe_4$): 26.9 (Me); 37.2 (C(3)); 62.2 (C(2)); 170.8 (C(1)).

N-*(2,3-Dibromo-2-methylpropanoyl)(*$^{15}N_2$*)urea* (**4**). Neat **3** (0.85 ml, 6.32 mmol) was added dropwise to a suspension of ($^{15}N_2$)urea (788 mg, 12.55 mmol) in MeCN (7.7 ml) at 50°. After 6 h, H_2O (3 ml) was added and the mixture allowed to react for an additional h. Then, the mixture was cooled, diluted with CH_2Cl_2 (40 ml), and extracted with H_2O (2 × 40 ml). After a back-extraction with CH_2Cl_2 (40 ml), the aq. soln. was neutralized with *Dowex 2* × *8* (OH^- form), evaporated, and dried *in vacuo*: 0.46 g (97.5%) of excess ($^{15}N_2$)urea, m.p. 136.2–137.2°. The combined org. solns. were extracted with 5% $NaHCO_3$ soln. (2 × 30 ml), dried (Na_2SO_4), and evaporated: 1.436 g (78%) of **4**. Colourless crystals. M.p. 132.5–135.5°. IR (KBr): 3425, 3325, 3220, 3140, 3040, 2980, 2920, 1675*s*, 1550, 1375, 1170, 1080. ^{1}H-NMR (300 MHz, $CO(CD_3)_2$, $SiMe_4$): 2.11 (*s*, Me); 4.10 (*d*, ^2J = 11, H_a–C(3)); 4.61 (*d*, ^2J = 11, H_b–C(3)); 6.85 (*d*, ^1J(N,H) = 92, 1 H of $^{15}NH_2$[2]); 7.94 (*d*, ^1J(N,H) = 91, 1 H of $^{15}NH_2$[2]); 9.60 (*d*, ^1J(N,H) = 91, $^{15}NH(CO)_2$[3]). ^{13}C-NMR (75 MHz, $CDCl_3$, $SiMe_4$): 26.8 (Me); 38.6 (C(3)); 58.9 (*d*, ^2J(N,C) = 8, C(2)); 155.0 (*dd*, ^1J(N,C) = 22, ^1J(N,C) = 16, CO(urea)); 171.2 (*d*, ^1J(N,C) = 13, C(1)). CI-MS (NH_3): 310 (22), 308 (46), 306 (23, $[M + NH_4]^+$), 293 (3), 291 (7), 289 (3, $[M + H]^+$), 148 (100, $[M + NH_4 - 2\,Br]^+$), 131 (42, $[M + H - 2\,Br]^+$), 104 (10), 87 (9).

($^{15}N_2$*)Thymine* (**5**). Pre-dried (P_2O_5, 16 h, high vacuum) **4** (1.379 g, 4.757 mmol) was suspended in abs. EtOH (10.5 ml) and the mixture heated to 50°. Then 1M NaOEt in EtOH (14.5 ml, 14.5 mmol of EtO^-) was added dropwise (→colourless precipitate). After 6 h at 50°, the mixture was acidified with 2M HCl (2.4 ml) and evaporated. The resulting solid was recrystallized (in 3 batches): 548 mg (85%) of **5**. IR (KBr): 3200, 3060, 2920, 1720*s*, 1670*s*, 1435, 1235, 1195, 835, 805. ^{1}H-NMR (300 MHz, CD_3SOCD_3, $SiMe_4$): 1.73 (*s*, Me); 7.26 (*d*, ^2J(N(1),H–C(6)) = 2, H–C(6)); 10.7 (br. *d*, ^1J(N,H) ≈ 85); 11.01 (*d*, ^1J(N,H) = 91). ^{13}C-NMR (75 MHz, CD_3SOCD_3, $SiMe_4$): 11.8 (Me); 107.6 (*d*, ^2J(N,C) = 6, C(5)); 137.8 (*d*, ^1J(N,C) = 11, C(6)); 151.4 (*dd*, ^1J(N,C) = 19, ^1J(N,C) = 16.5, C(2)); 165.0 (*d*, ^1J(N,C) = 9, C(4)). ^{15}N-NMR (41 MHz, ^{1}H-broadband-decoupled, 0.1M sodium phosphate buffer, pH 7.0, 10% (*v*/*v*) D_2O, 25°, internal $^{15}NH_4Cl$): 110.82 (*d*, ^2J(N,N) = 2.5, N(1)); 137.00 (*d*, ^2J(N,N) = 2.5, N(3)). ^{15}N-NMR (41, MHz, CD_3SOCD_3, 25°, internal $^{15}NH_4Cl$): 103.9 (br. *s*, N(1)); 131.7 (br. *d*, ^1J(H,N) = 76, N(3)). EI-MS (70 eV): 128 (100, $[(^{15}N_2)M]^+$), 127 (0.16, $[(^{15}N_1,^{14}N_1)M]^+$), 84 (10, $[M - CO^{15}NH]^+$), 83 (8), 56 (75), 55 (36).

*2′,3′,5′-Tris-*O*-benzoyl-5-methyl(*$^{15}N_2$*)uridine* (**6**). A suspension of **5** (295.1 mg, 2.3 mmol) in hexamethyldisilazane ($(Me_3Si)_2NH$; 4.9 ml) was refluxed under Ar for 16 h. After evaporation, the oil was redissolved in abs. 1,2-dichloroethane (3 ml) and added to a soln. of 1-*O*-acetyl-2,3,5-tris-*O*-benzoyl-β-D-ribofuranose (1.501 g, 2.98 mmol) in 1,2-dichloroethane (5 ml). $SnCl_4$ (0.30 ml, 2.55 mmol) was rapidly added to the soln. at r.t. (H_2O bath) and the yellowish mixture stirred overnight. The soln. was poured into 5% aq. $NaHCO_3$, $CHCl_3$ (50 ml) was added and the mixture filtered over *Celite*. After separation of the phases, the org. soln. was dried (Na_2SO_4) and evaporated: 1.5 g of crude yellowish foam. FC (SiO_2, 2% MeOH/CH_2Cl_2) gave 909.2 mg (69%) of pure amorphous **6**. ^{1}H-NMR (300 MHz, $CDCl_3$, $SiMe_4$): 1.6 (*s*, Me–C(5)); 4.65 (*dd*, ^3J = 3.5, ^2J = 12, H_a–C(5′)); 4.70 (*td*, ^3J = 3.5, 2.5, H–C(4′)); 4.89 (*dd*, ^3J = 2.5, ^2J = 12, H_b–C(5′)); 5.76 (*td*, ^3J = 6, ^3J(N(1),H–C(2′)) = 1.5, H–C(2′)); 5.92 (*dd*, ^3J = 3.5, 6, H–C(3′)); 6.44 (*d*, ^3J = 6, H–C(1′)); 7.16 (br. *s*, H–C(6)); 7.3–7.65, 7.8–8.0, 8.1–8.2 (*m*, 3 Ph); 8.74 (*dt*, ^1J(N(3),H) = 91, *J* = 2, H–N(3)). ^{13}C-NMR (75 MHz, $CDCl_3$, $SiMe_4$, assignments according to a ^{1}H/^{13}C-HETCOR): 12.08 (Me); 63.91 (C(5′)); 71.42 (C(3′)); 73.35 (C(2′)); 80.60 (C(4′)); 86.87 (*d*, ^1J(N,C) = 13.5, C(1′)); 112.22 (C(5)); 128–130 (C_o,C_m,C_p); 133.68, 133.74 (C_{ipso}); 134.78 (*d*, ^1J(N,C) = 12, C(6)); 150.22 (*t*, ^1J(N,C) ≈ 12, C(2)); 163.29 (*d*, ^1J(N,C) ≈ 12, C(4)); 165.30, 165.38, 165.96 (Ph*C*O). FAB$^+$-MS (nitrobenzyl alcohol): 573 (10, $[M + H]^+$), 445 (28, $[M - B]^+$), 201 (9), 129 (2, $[B + 2H]^+$), 105 (100, $PhCO^+$), 77 (15).

---

[2]) Exchanges slowly with D_2O.

[3]) Exchanges rapidly with D_2O.

5-Methyl($^{15}N_2$*)uridine* (**7**). A suspension of **6** (872.1 mg, 1.52 mmol) in abs. MeOH (38 ml) at r.t. was treated with 353 mM NaOMe in MeOH (10 ml, 3.53 mmol). After 2 h, the clear soln. was neutralized with *Dowex 50W* × *8* (H^+ form) and evaporated: 354.8 mg (89.5%) of **7**. TLC (AcOEt/MeOH/H_2O 4:1:0.4): R_f 0.52. M.p. 176.0–180.0°. $[\alpha]_D^{25} = -12.8$ (*c* = 1.1, MeOH). ^{1}H-NMR (300 MHz, CD_3SOCD_3, $SiMe_4$): 1.77 (*s*, Me–C(5)); 3.54 (*ddd*, ^3J(OH,CH_2(5′)) = 5.2, ^2J = 12, ^3J = 3.5, H_a–C(5′)); 3.64 (*ddd*, ^3J(OH,CH_2(5′)) = 5.2, ^2J = 12, ^3J = 3.5, H_b–C(5′)); 3.82 (*q*, ^3J = 3.5, H–C(4′)); 3.97 (*dd*, ^3J(OH–C(3′),H–C(3′)) = 4.9, ^3J = 4.2, H–C(3′)); 4.03 (*dd*, ^3J(OH–C(2′),H–C(2′)) = 6, ^3J = 5, H–C(2′)); 5.04 (*d*, ^3J(H–C(3′),OH–C(3′)) = 4.9, OH–C(3′)); 5.09 (*t*, ^3J(CH_2(5′),OH–C(5′)) = 5.2, OH–C(5′)); 5.31 (*d*, ^3J(H–C(2′),OH–C(2′) = 5.8, OH–C(2′)); 5.78 (*d*, ^3J = 5.5, H–C(1′)); 7.73 (*s*, H–C(6)); 8.74 (br. *d*, ^1J(N(3),H) = 85, H–N(3)). FAB^+-MS (glycerine): 262 (7, [($^{15}N_2$)*M* + 2H]$^+$), 261 (37, [($^{15}N_2$)*M* + H]$^+$), 129 (100, [($^{15}N_2$)B + 2H]$^+$), 128 (7, [($^{15}N_2$)B + H]$^+$), 127 (4, [($^{15}N_2$)B]$^+$).

*2′,3′-*O*-Isopropylidene-5-methyl(*$^{15}N_2$*)uridine* (**8**). Conc. H_2SO_4 (6.7 µl, 0.12 mmol) was added to a well stirred suspension of **7** (315 mg, 1.21 mmol) and anh. $CuSO_4$ (575 mg, 3.6 mmol) in acetone (30 ml). After 4 d, the suspension was filtered through a *G-3* frit containing a thin (1–2 mm) layer of $Ca(OH)_2$ into a vessel containing more (1 g) $Ca(OH)_2$. The upper layer was washed with 5 portions of acetone and the combined yellow suspension stirred for 1 h. Filtration through *Celite* and evaporation furnished quantitatively a yellow oil (TLC (AcOEt/MeOH/H_2O 4:1:0.2): R_f 0.67) which was used in the following reaction without purification. The spectroscopic characterization was carried out with unlabelled material. ^{1}H-NMR (300 MHz, $CDCl_3$, $SiMe_4$): 1.32, 1.54 (2*s*, Me_2C); 1.88 (*s*, Me–C(5)); 3.1 (br. *s*, OH); 3.81 (*dd*, ^2J = 12, ^3J = 3.2, H_a–C(5′)); 3.92 (*dd*, ^2J = 12, ^3J = 2.4, H_b–C(5′)); 4.27 (*q*, ^3J = 3, H–C(4′)); 4.98 (*dd*, ^3J = 3, 6, H–C(3′)); 5.03 (*dd*, ^3J = 3, 6, H–C(2′)); 5.51 (*d*, ^3J = 3, H–C(1′)); 7.14 (*s*, H–C(6)); 8.9 (br. *s*, H–N(3)).

*2,5′-Anhydro-2′,3′-*O*-isopropylidene-5-methyl(*$^{15}N_2$*)uridine* (**9**). Fresh PPh_3 (952.1 mg, 3.63 mmol) and **8** (1.2 mmol) were dried *in vacuo*. A yellow THF soln. thereof (25 ml) was treated with diethyl azodicarboxylate (DEAD; 570 µl) under Ar. After 1 min, a precipitate (**9**) appeared, and after 5 min, the reaction was complete. The liquid was decanted and the precipitate boiled in fresh THF and filtered several times to yield a 1st portion of colourless **9** (167.8 mg). The combined mother liquors were evaporated (1.6 g) and purified by CC (10% MeOH/$CHCl_3$; TLC: R_f 0.45) and crystallization to yield a 2nd portion of colourless **9** (112.7 mg). The combined second mother liquors were evaporated and purified by FC using a step gradient (AcOEt/MeOH/H_2O 9:1:0.1, 6:1:0.1, and 4:1:0.1) to yield a 3rd portion of colourless **9** (9.5 mg). TLC (AcOEt/MeOH/H_2O 4:1:0.2): R_f 0.5. Total yield: 257.2 mg (75%). Unlabelled material was used for the spectroscopic characterization. IR (KBr): 1650*s*, 1550*s*. ^{1}H-NMR (300 MHz, $CDCl_3$, $SiMe_4$): 1.36, 1.53 (2*s*, Me_2C); 2.00 (*s*, Me–C(5)); 4.15 (*d*, ^2J = 13, H_a–C(5′)); 4.45 (*dd*, ^2J = 13, ^3J = 1.5, H_b–C(5′)); 4.67 (br. *s*, H–C(4′)); 4.88 (*d*, ^3J = 5.5, H–C(3′)); 4.94 (*d*, ^3J = 5.5, H–C(2′)); 5.34 (*s*, H–C(1′)); 7.18 (*s*, H–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$, $SiMe_4$): 13.36 (CH_3–C(5)); 24.44, 26.01 (Me_2C); 74.18 (C(5′)); 81.90, 84.75, 85.74 (C(2′), C(3′), C(4′)); 98.66 (C(1′)); 113.25 (C(5)); 119.96 (Me_2C); 137.42 (C(6)); 156.39 (C(2)); 171.60 (C(4)). FAB^+-MS (nitrobenzyl alcohol): 282 (14), 281 (100, [($^{14}N_2$)*M* + H]$^+$).

*2′,3′-*O*-Isopropylidene-5-methyl(*$^{15}N_2$*)[*$O^{2'}$-$^{17/18}O_2$*]uridine* (**10**). $H_2[^{17/18}O]$ (1.0 ml) was cooled down to −180° (liq. N_2) using a closed vessel, a rubber septum, and a balloon filled with Ar. After a liquid layer of Ar formed over the frozen water, Na (26 mg, 1.13 mmol) was quickly added in two portions. The reaction was efficiently controlled by gradually melting and re-freezing the water over the liquid N_2 bath. Na reacted only with liquid, not with frozen water. The liquid Ar layer protected the Na from residual air and, by evaporating, acted as a rapidly responding heat transfer medium. The balloon acted as an Ar reservoir and elastic volume for H_2 uptake. After all Na was oxidized to Na[$^{17/18}$O]H, the soln. was warmed to r.t., and **9** (200 mg, 0.71 mmol) was added. After stirring at r.t. for 40 min, the suspension was heated to 50° and gently stirred for 3.5 h (TLC (AcOEt/MeOH/H_2O 4:1:0.2): practically complete conversion). After another 45 min at 50°, the mixture was cooled to r.t. After 16 h, the soln. was neutralized with *Dowex 50W* × *8* (450 mg, 20–50 mesh, H^+ form), whereupon some residual turbidness dissolved. The whole mixture was carefully lyophilized in silanized glassware, and the regenerated labelled $H_2[^{17/18}O]$ (1.065 g) was trapped in a liq.-N_2 bath. The *Dowex* resin was liberated from the product by washing it with acetone. Evaporation of the solvent furnished **10** as a brown oil that was used in the following reaction without further purification (TLC (AcOEt/MeOH/H_2O 4:1:0.2): R_f 0.5). FAB^+-MS (nitrobenzyl alcohol): 303 (53.3, [($^{18}O_1$,$^{16}O_1$)*M* + H]$^+$ + [($^{17}O_1$,$^{16}O_1$)*M* + 2H]$^+$), 302 (66.0, [($^{18}O_1$,$^{16}O_1$)*M*]$^+$ + [($^{17}O_1$,$^{16}O_1$)*M* + H]$^+$ + [($^{16}O_2$)*M* + 2H]$^+$), 301 (61.3, [($^{17}O_1$,$^{16}O_1$)*M*]$^+$ + [($^{16}O_2$)*M* + H]$^+$), 273 (16), 173 (62), 131 (73.3, [($^{18}O_1$,$^{16}O_1$)B + 2H]$^+$), 130 (94, [($^{18}O_1$,$^{16}O_1$)B + H]$^+$ + [($^{17}O_1$,$^{16}O_1$)B + 2H]$^+$), 129 (100, [($^{18}O_1$,$^{16}O_1$)B]$^+$ + [($^{17}O_1$,$^{16}O_1$)B + H]$^+$ + [($^{16}O_2$)B + 2H]$^+$).

*2′,3′-*O*-Isopropylidene-5′-*O*-[(4-methoxyphenyl)diphenylmethyl]-5-methyl(*$^{15}N_2$*)[*$O^{2'}$-$^{17/18}O_2$*]uridine* (**11**). Crude **10** (0.70 mmol) was 3× co-evaporated with abs. pyridine and kept under Ar in pyridine (10 ml), before monomethoxytrityl chloride (366 mg, 1.19 mmol) was added in 3 portions (1.1 equiv. at r.t.; after 20 h, 0.2 equiv. at 45°; after 22 h, 0.4 equiv. at r.t.). Then MeOH (10 ml) was added to the soln. After 15 min, pyridine was evaporated,

the residual oil dissolved in AcOEt, and the soln. extracted with 5% $NaHCO_3$ soln. (3 ×), dried (Na_2SO_4), and evaporated. FC (SiO_2, $CHCl_3$, then 3%, 5%, and 8% MeOH/$CHCl_3$ (step gradient)): 43 mg of unreacted **10** (TLC (5% MeOH/CH_2Cl_2): R_f 0.25) and 258.9 mg (80.7%) of pure, solid **11** (TLC (5% MeOH/CH_2Cl_2): R_f 0.5, bright yellow spot upon treatment with 5% H_2SO_4/EtOH).

2′,3′-O-Isopropylidene-5-methyl($^{15}N_2$*)[*O^2,O^4-$^{17/18}O_2$*]uridine* (**14**). A suspension of 3-nitro-1*H*-1,2,4-triazole (128.3 mg, 1.13 mmol) and diphenyl chlorophosphate (233 µl, 1.13 mmol) in MeCN (2.25 ml) at 0° was treated with Et_3N (313.6 µl, 2.25 mmol) under Ar (→clear soln., then orange and colourless precipitate). After 0.5 h at 0°, a soln. of **11** (258.9 mg, 0.45 mmol) in MeCN (1.5 ml) was added and the cooling bath removed. After 2 h at r.t. (TLC (5% MeOH/$CHCl_3$): almost complete conversion of **11** (R_f 0.5) into **12** (R_f 0.6)), the suspension was filtered into H_2O (0.4 ml), and the solvents were evaporated to yield a brown-red tar which was redissolved in CH_2Cl_2 (10 ml) and extracted with 5% $NaHCO_3$ soln. (10 ml) and H_2O (2 × 10 ml). The org. soln. was dried ($MgSO_4$) and evaporated: 292 mg of crude **12**. A soln. thereof in abs. THF (2 ml) was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 104.4 µl, 0.68 mmol) and $H_2[^{17/18}O]$ (16.2 µl, 0.85 mmol). After stirring for 14 h at r.t., the soln. was poured into 0.5M NH_4Cl (5 ml) and extracted with CH_2Cl_2 (3 × 20 ml). The org. soln. was extracted with 5% $NaHCO_3$ soln. (2 × 20 ml), dried ($MgSO_4$), and evaporated: 293 mg of crude oil (**13**). Then 80% aq. AcOH (5 ml) was added and the soln. stirred for 1 h at 60°. The mixture was lyophilized and purified by FC (15 g SiO_2, AcOEt/MeOH/H_2O 4:1:0.05) to give TLC-pure, oily **14**. A THF soln. thereof (3 ml) was split in a 1-ml and a 2-ml portion. The latter was treated with hexane to precipitate the product. Evaporation and drying *in vacuo* yielded 53 mg (0.175 mmol) of yellowish, amorphous powder. The colour disappeared after reversed-phase HPLC purification (*Knauer* column, 16 mm i.d.; *Spherisorb ODS II*, 5 µm, 50 Å; flow rate 14 ml min^{-1}, column temp. 40°, detection at 270 nm; eluent: H_2O (*A*), H_2O/MeCN 7:3 (*B*), *i.e.* 0→67% *B* in 15 min, stay at 67% *B* for 7 min; t_R 16.8 min). ^{15}N-NMR (41 MHz, ^{1}H-broadband-decoupled, $CDCl_3$, 25°, external $Me^{15}NO_2$[4])): −239.71 (*d*, ^2J(N,N) = 2.5, N(1)); −225.61 (*d*, ^2J(N,N) = 2.5, N(3)). ^{17}O-NMR (54 MHz, $CHCl_3$, 45°, external 1,4-dioxane (neat)): 252.9 (br. *s*, $w_{½}$ = 910[5]), O–C(2)); 336.6 (br. *s*, $w_{½}$ = 810[5]), O–C(4)). FAB$^+$-MS (nitrobenzyl alcohol): 305 (12.8, $[(^{18}O_2)M + H]^+$), 304 (35.3, $[(^{18}O_2)M]^+ + [(^{18}O_1,^{17}O_1)M + H]^+ + [(^{17}O_2)M + 2H]^+ + [(^{18}O_1,^{16}O_1)M + 2H]^+$), 303 (79.0, $[(^{18}O_1,^{17}O_1)M]^+ + [(^{17}O_2)M + H]^+ + [(^{18}O_1,^{16}O_1)M + H]^+ + [(^{17}O_1,^{16}O_1)M+2H]^+$), 302 (80.5, $[(^{17}O_2)M]^+ + [(^{17}O_1,^{16}O_1)M + H]^+ + [(^{16}O_2)M + 2H]^+$), 301 (63.0, $[(^{17}O_1,^{16}O_1)M]^+ + [(^{16}O_2)M + H]^+$), 273, (17), 173 (80, $[M - B]^+$), 133 (16.9, $[(^{18}O_2)B + 2H]^+$), 132 (37.3, $[(^{18}O_2)B + H]^+ + [(^{18}O_1,^{17}O_1)B + 2H]^+$), 131 (97.7, $(^{18}O_2)B^+ + [(^{18}O_1,^{17}O_1)B + H]^+ + [(^{17}O_2)B + 2H]^+ + [(^{18}O_1,^{16}O_1)B + 2H]^+$), 130 (100.0, $[(^{18}O_1,^{17}O_1)B]^+ + [(^{17}O_2)\ B + H]^+ + [(^{18}O_1,^{16}O_1)B + H]^+ + [(^{17}O_1,^{16}O_1)B + 2H]^+$), 129 (90.1, $[(^{17}O_2)B]^+ + [(^{18}O_1,^{16}O_1)B]^+ + [(^{17}O_1,^{16}O_1)B + H]^+ + [(^{16}O_2)B + 2H]^+$).

5-Methyl($^{15}N_2$*)[*O^2,O^4-$^{17/18}O_2$*]uridine* (**15**). Approximately one third of the previously synthesized material (**14**) in THF (1 ml) was treated with 90% aq. CF_3COOH (1 ml) during 1 h at r.t. to form quantitatively **15** (TLC (AcOEt/MeOH/H_2O 4:1:0.4): R_f 0.5) in a bright orange soln. Evaporation of the sovents at 40° *in vacuo*, repeated co-evaporation with THF, precipitation into hexane, decanting, and drying yielded 30.7 mg (0.117 mmol) of TLC-pure **15** as a yellowish solid. Combined chemical yield of both **14** (*ca.* 2/3) and **15** (*ca.* 1/3) relative to **11**: 68%. The colour of **15** disappeared upon desalting by reversed-phase HPLC after the NMR measurements (column, packing, and other conditions as for **14**, except for eluent (0→10% *B* in 9 min, stay at 10% *B* for 3 min); t_R 9.4 min, confirmed by an unlabelled reference sample). ^{15}N-NMR (41 MHz, ^{1}H-broadband-decoupled, 0.1M aq. sodium phosphate buffer, pH 7.0, 5% (*v*/*v*) D_2O, 25°, internal $^{15}NH_4Cl$[4])): 122.37 (*d*, ^2J(N,N) = 2.5, N(1)); 136.51 (*d*, ^2J(N,N) = 2.5, N(3)). ^{17}O-NMR (0.1M aq. sodium phosphate buffer, pH 7.0, 45°, external, 1,4-dioxane (neat)): 231.6 (br. *s*, $w_{½} \approx 790$[5]), O–C(2)); 296.8 (br. *s*, $w_{½} \approx 940$[5]), O–C(4)). FAB$^+$-MS (glycerine): 264 (12.1, $[(^{18}O_2)M]^+$), 263 (24.5, $[(^{18}O_1,^{17}O_1)M]^+$), 262 (25.6, $[(^{17}O_2)M]^+ + [(^{18}O_1,^{16}O_1)M]^+$), 261 (19,9, $[(^{17}O_1,^{16}O_1)M]^+$), 133 (20.2, $[(^{18}O_2)B + 2H]^+$), 132 (17.5, $[(^{18}O_2)B + H]^+ + [(^{18}O_1,^{17}O_1)B + 2H]^+$), 131 (100, $[(^{18}O_2)B]^+ + [(^{18}O_1,^{17}O_1)B + H]^+ + [(^{17}O_2)B + 2H]^+ + [(^{18}O_1,^{16}O_1)B + 2H]^+$), 130 (40.5, $[(^{18}O_1,^{17}O_1)B]^+ + [(^{17}O_2)B + H]^+ + [(^{18}O_1,^{16}O_1)B + H]^+ + [(^{17}O_1,^{16}O_1)B + 2H]^+$), 129 (30.4, $[(^{17}O_2)B]^+ + [(^{18}O_1,^{16}O_1)B]^+ + [(^{17}O_1,^{16}O_1)B + H]^+ + [(^{16}O_2)B + 2H]^+$).

REFERENCES

[1] J.Seres, K. Daroczi, P. Seres, B. Köszegi, DDR Pat., DD 222 591 A5, 1985.

[2] H. Vorbrüggen, K. Krolikiewicz, B. Bennua, *Chem. Ber.* **1981,** *114,* 1234; H. Vorbrüggen, G. Höfle, *ibid.* **1981,** *114,* 1256.

[3] R. T. Solsten, J. A. McCloskey, K. H. Schram, *Nucleosides Nucleotides* **1980,** *1,* 57.

[4] B. F. L. Li, C. B. Reese, P. F. Swann, *Biochemistry* **1987,** *26,* 1086.

[4]) δ(ext. neat $Me^{15}NO_2$) = 360.7 ppm + δ(int. aq. $^{15}NH_4Cl$).

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