230. Synthesis of 5-Methyluridine and Its 5′-Mercapto-, 2-Amino-, and 4′,5′-Unsaturated Analogues

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Summary

The stereospecific *cis*-hydroxylation of 1-(2,3-dideoxy-β-D-glyceropent-2-enofuranosyl)thymine (**1**) into 1-β-D-ribofuranosylthymine (**2**) by osmium tetroxide is described. Treatment of 2′,3′-*O*,*O*-isopropylidene-5-methyl-2,5′-anhydrouridine (**8**) with hydrogen sulfide or methanolic ammonia afforded 5′-deoxy-2′,3′-*O*,*O*-isopropylidene-5′-mercapto-5-methyluridine (**9**) and 2′,3′-*O*,*O*-isopropylidene-5-methyl-isocytidine (**10**), respectively. The action of ethanolic potassium hydroxide on 5′-deoxy-5′-iodo-2′,3′-*O*,*O*-isopropylidene-5-methyluridine (**7**) gave rise to the corresponding 1-(5-deoxy-β-D-erythropent-4-enofuranosyl)5-methyluracil (**13**) and 2-*O*-ethyl-5-methyluridine (**14**).

The hydrogenation of **2** and its 2′,3′-*O*,*O*-isopropylidene derivative **4** over 5% Rh/Al_2O_3 as catalyst generated diastereoisomers of the corresponding 5-methyl-5,6-dihydrouridine (**17** and **18**).

The 1-β-D-ribofuranosylthymine (5-methyluridine (**2**)) has received a great deal of attention as a TψC loop unit of transfer-ribonucleic acids. Possible utilities of this unusual nucleic acid constituent as a biochemical and biogenetic tool directed our search toward its synthesis and conversion into the hitherto unknown 5′-mercapto, 2-amino-, 4′,5′-unsaturated and 5,6-dihydro-analogues.

The literature includes various methods for the preparation of ribosylthymine (**2**). Thus, the acyl derivatives of **2** were generated by the condensation of dithyminylmercury- [1], 2,4-di-*O*-ethyl- [2], or 2,4-di-*O*-trimethylsilyl- [3] [4] -thymine with 2′,3′,5′-tri-*O*-benzoyl- (or acetyl) -D-ribofuranosyl halides. The synthesis of **2** was also accomplished by the regiospecific formylation of uridine, followed by the catalytic hydrogenation of the resulting 5-hydroxymethyluridine (**3**) [5] [6]. To our knowledge, there have been no reports dealing with the *cis*-hydroxylation of 2′,3′-unsaturated 5-methylpyrimidine nucleosides, except for 1-(3-cyclopent-1-yl)thymine which was converted into the 3,4-diol [7] when treated with osmium tetroxide. The application of a similar approach to the synthesis of **2** required the known 1-(2,3-dideoxy-β-D-glyceropent-2-enofuranosyl)thymine (**1**), which was easily prepared from 1-(2-deoxy-3,5-epoxy-β-D-threopentofuranosyl)thymine [8] according to the procedure described by *Horwitz et al.* [9].

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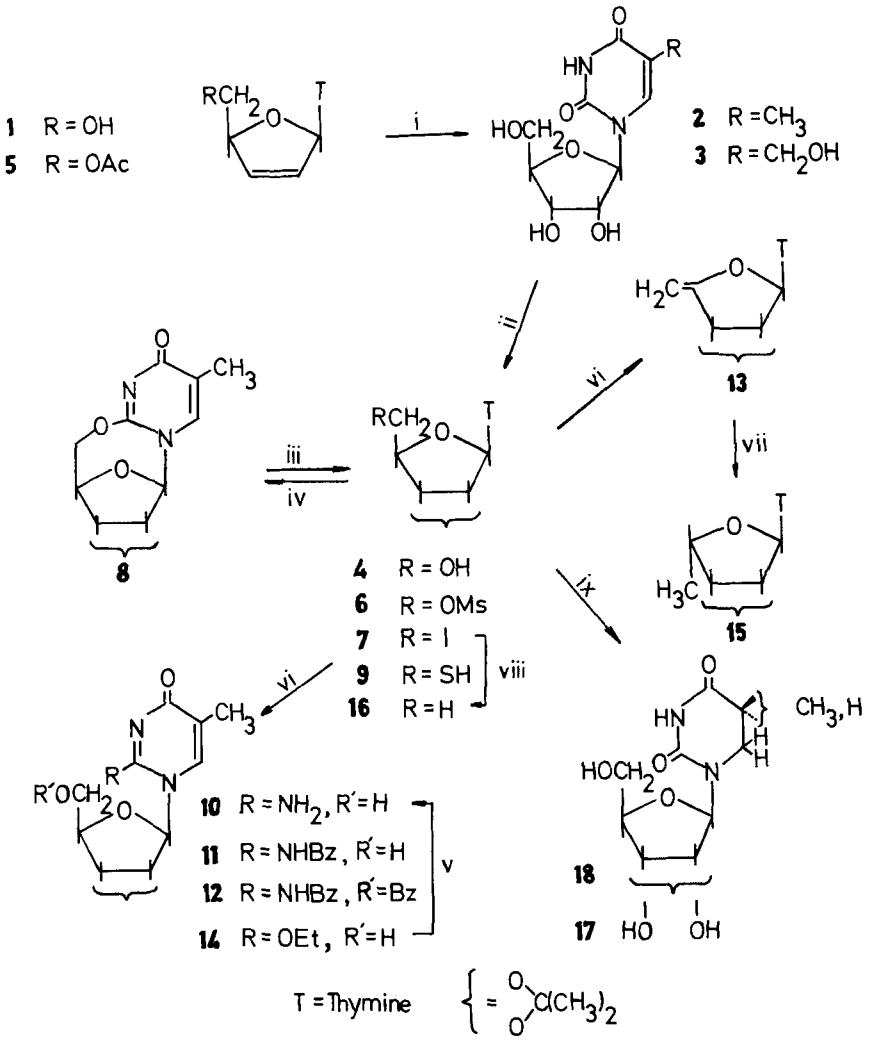

i: OsO_4/C_6H_6-DMF/py; ii: $(CH_3)_2CO/H_2SO_4/CuSO_4$; iii: AgOAc/MeOH; iv: H_2S/DMF/Et_3N; v: NH_3/MeOH; vi: KOH/EtOH; vii: [H_2], 5% Pd/C in EtOH; viii: [H_2], 5% Pd/C in 1 N NaOH/EtOH; ix: 5% $Rh/Al_2O_3/H_2O$.

The *cis*-hydroxylation of 3'-deoxy-2'-thymidinene **1** on the less hindered side using osmium tetroxide as oxidant [10] (path i) afforded **2**, identical with the authentic sample, unambiguously prepared via 5-hydroxymethyluridine [5] (**3**). Moreover, the 2',3'-*O*,*O*-isopropylidene-5-methyluridine (**4**) (path ii), derived from the hydroxylated thymidinene **1**, showed the same spectroscopic (IR., NMR.) characteristics as those reported elsewhere [11] [12]. It is worth noting that the treatment of thymidinene **1** with sodium chlorate/osmium tetroxide [13] as well as with potassium permanganate [14] afforded a mixture of unidentified products and low yields of the expected glycol **2**.

An attempt to convert thymidinene **1** into the *cis*-β-glycol stereoisomer by the silver acetate/iodine method [15] resulted in the thymine formation. In evaluating the factors inducing this glycosidic bond cleavage, we found that the bromination of 1-(5-*O*-acetyl-2,3-dideoxy-β-D-glyceropent-2-enofuranosyl)thymine (**5**) in CH_2Cl_2 under mild conditions led to a similar cleavage and to the thymine formation in a nearly quantitative yield. Thus, the glycosidic bond cleavage during the silver acetate/iodine oxidation can be explained by invoking the halogenation step as the most decisive one.

The synthesis of 2′,3′-*O*,*O*-isopropylidene-5′-*O*-methanesulfonyl-5-methyluridine (**6**) and its conversion into 5′-deoxy-5′-iodo-2′,3′-*O*,*O*-isopropylidene-5-methyluridine (**7**) on treatment with sodium iodide [16] were accomplished in high yields. The latter smoothly cyclized into 2′,3′-*O*,*O*-isopropylidene-5-methyl-2,5′-anhydrouridine (**8**) using silver acetate (path iii) as condensing agent [17]. Reaction of the thus obtained anhydro-compound **8** with hydrogen sulfide (path iv) or methanolic ammonia (path v) furnished the hitherto unknown 5′-deoxy-2′,3′-*O*,*O*-isopropylidene-5′-mercapto-5-methyluridine (**9**) and 2′,3′-*O*,*O*-isopropylidene-5-methyl-isocytidine (**10**), respectively, the latter being characterized as 2-*N*-benzoyl- (**11**) and 2-*N*,5′-*O*-dibenzoyl- (**12**) 2′,3′-*O*,*O*-isopropylidene-5-methyl-isocytidines. The NMR. spectrum of the 5′-mercapto analogue **9** retained the characteristics of 2-oxo-compounds but caused a marked upfield shift of the H_2–C(5′) to δ 3.10 ppm and HS–C(5′) at 1.21 ppm, placing them in the range of the signals of the primary mercapto compounds. The formation of **9** in 85% yield confirmed highly selective nucleophilic attack of the weak nucleophilic HS^- species at the position C(5′) of 5-methyl-2′,5′-anhydrouridine **8**. An indication that electronic effect of CH_3–C(5) in **8** could contribute to this specific ring opening is provided by a comparison of the results obtained with the unsubstituted 2,5′-anhydrouridine [18] [19] which underwent diverse reactions yielding several products, among them 2-*C*-thiouridine as the expected one. In contrast to the HS^- attack, ammonia as nucleophile overcame electron denisty barriers in the thymine ring system affording the 2-amino analogue **10**, idenpendently prepared from 2-*O*-ethyl-2′,3′-*O*,*O*-isopropylidene-5-methyluridine (**14**) (path v).

We recently reported the synthesis of isocytidine analogues bearing 4′,5′-double bond as part of our interest for nucleoside antibiotics [20]. The analogous 1-(5-deoxy-2,3-*O*,*O*-isopropylidene-β-D-erythropent-4-enofuranosyl)-5-methyluracil (**13**) was prepared from the 5′-iodo compound **7** by reaction with ethanolic potassium hydroxide (path vi) and evidenced in the NMR. spectrum by a characteristic pair of doublets (J = 1.8 Hz) at δ 4.56 and 4.38 ppm corresponding to the 4′-exo-methylene group. It is interesting that besides the dehydroiodation of **7** into **13** a concomitant ring opening of the intermediary anhydro compound **8** proceeded to 2-*O*-ethyl-5-methyluridine derivative **14**, the latter being independently prepared from the anhydro-compound **8** (path vi) in high yields. The NMR. spectrum evidenced structure **14** by the characteristic quadruplet at δ 4.35 ppm and triplet at δ 1.31 ppm, attributed to the *O*-ethyl group and by the resonances at δ 7.72 and 1.78 ppm for the H–C(6) and H_3C–C(5), respectively, being in good accordance with those of the 2,3-double bond bearing anhydro structure **8**.

The hydrogenation of the 4′,5′-unsaturated compound **13** (path vii) stereo-

specifically proceeded into 1-(5-deoxy-2,3-*O*, *O*-isopropylidene-*α*-L-lyxopentofuranosyl)5-methyluracil (**15**) by the procedure described in the conversion of the analogous uridine derivative [21]. The diastereoisomeric 5′-deoxy-2′,3′-*O*, *O*-isopropylidene-5-methyluridine (**16**) was prepared from the 5′-iodo compound **7** by catalytic hydrogenation in a basic ethanolic solution [22] (path viii). The NMR. spectra of the compounds **15** and **16** clearly evidenced their diastereoisomeric features showing anomeric C(1′)-proton resonances at δ 5.31 and 5.63 ppm, C(4′)-proton resonances at δ 4.57 and 4.17 ppm, and C(4′)-methyl resonances at δ 1.33 and 1.40 ppm, respectively.

The occurrence of 5-methyl-5,6-dihydrouridine (**17**) (8.1 mol%) in chromosomal RNA associated with the chromosomes of aschites tumor cells [23] stimulated also our search toward its stereochemical features. Previous reports have shown that the ribosylthymine **2** and its tri-*O*-acetyl derivative can be simply reduced to the corresponding 5-methyl-5,6-dihydrouridines [23] [24] using 5% Rh/Al_2O_3 as catalyst [25] (path ix). In contrast to this finding, our attempt to hydrogenate compound **2** and its 2′,3′-*O*, *O*-isopropylidene derivative **4** under the mentioned conditions resulted in a mixture of diastereoisomeric 5-methyl-5,6-dihydrouridines (**17**) and a mixture of diastereoisomeric 2′,3′-*O*, *O*-isopropylidene-5-methyl-5,6-dihydrouridines (**18**), respectively.

The examination of the NMR. spectrum of **18** showed that this sample was actually a mixture of the two diastereoisomers being formed in an approximate ratio of 6:4. The major component revealed the more intense doublet at δ 5.46 ppm attributed to the anomeric H–C(1′) than that at δ 4.68 ppm belonging to another isomer. The same relative intensities of the doublets at δ 1.27 and 1.25 ppm were found for resonances of H_3C–C(5). However, conditions for the preparative separation and the configuration of the pure isomers **18** remained to be examined as part of our studies on the chemistry of dihydropyrimidine nucleosides [26–28]. A preliminary separation of stereoisomeric **17** ($[\alpha]_D^{29} = -32°$ ($c = 1$, methanol)) by fractional crystallization in methanol/ether afforded a pure isomer ($[\alpha]_D^{26} = +25.9°$ ($c = 0.85$, methanol)).

Experimental Part

General. The same techniques and apparatus were used as described previously [28]. In addition, optical rotations were measured in acetone and separations of products by prep. TLC., developed in CH_2Cl_2/methanol 10:1, recovery with acetone, unless otherwise stated. Mass spectra were measured with a *Varian* MAT CH7 spectrometer.

5-Methyluridine (**2**). – a) To a solution of 1-(2,3-dideoxy-*β*-D-glyceropent-2-enofuranosyl)thymine [8] (**1**, 112 mg, 0.5 mmol) in anhydrous DMF (3 ml) and pyridine (0.08 ml), protected from moisture, osmium tetroxide (163 mg, 0.64 mmol) in benzene (2 ml) was added dropwise and then stirred at RT. for additional 90 min. The mixture was treated with hydrogen sulfide (gas), and then the precipitate filtered off. The filtrate was evaporated i.V. to dryness and separated by prep. TLC. (three developments). Crystalline **1** was obtained (80 mg, 74%), Rf 0.16, m.p. 180–181° (methanol/acetone/hexane), (m.p. 183–185° (corr.) [1], $[\alpha]_D^{24} = -8.6°$ ($c = 0.7$, H_2O), ($[\alpha]_D^{31} = -10°$ ($c = 2$, H_2O) [29]), the NMR. identical with that reported earlier [30]. – UV.: 267 (3.89). – IR.: 3438, 3380, 3288, 3040, 2920, 1713, 1689, 1658 br., 1647 sh., 1044.

$C_{10}H_{14}N_2O_6$ (258.23) Calc. C 46.50 H 5.46 N 10.85% Found C 46.22 H 5.70 N 10.97%

b) To a solution of **1** (200 mg, 0.89 mmol) in methanol/water 1:1 (2 ml), sodium chlorate (123 mg, 1.16 mmol) and osmium tetroxide (2.7 mg) were added. The mixture was stirred at RT. for 29 h, then

diluted with water (5 ml), and passed through a column of *Amberlite* IR-4B (acetate-form, *ca.* 7 ml). The water eluate was concentrated on a 10 ml volume and then applied on a column of *Dowex* X8 (H-form, *ca.* 7 ml), water eluates evaporated i.V. to dryness (220 mg), and the residue dissolved in methanol. Prep. TLC. (CH_2Cl_2/methanol 4:1, recovery with methanol) afforded the product **2** (50 mg, 22%), identical (IR. and NMR. spectra) with that obtained under a).

c) To a solution of **1** (112 mg, 0.5 mmol) in acetone (12.5 ml), potassium permanganate (118 mg, 0.75 mmol) in water (10 ml) was added. The mixture was stirred at RT. for 1 h, the precipitate filtered off, and the filtrate evaporated to dryness (130 mg). Prep. TLC. (five developments) afforded the product **2** (27 mg, 23%), identical with that obtained under a). The starting material, Rf 0.33 (8 mg), and thymine, Rf 0.27 (13 mg, 22%), as by products were also isolated.

2′,3′-O,O-Isopropylidene-5-methyluridine [11] (**3**). Using the procedure reported earlier [17] 5-methyluridine (**2**; 110 mg, 0.426 mmol) in anhydrous acetone (2.5 ml), was treated with desiccated cuprous sulfate (210 mg) and conc. sulfuric acid (0.0025 ml). Prep. TLC. separated the product **3** (95 mg, 75%), Rf 0.5, m.p. 74–75° (ethyl acetate/hexane) (as hydrate: shranks at 75° and melts at 112° [11]), $[\alpha]_D^{25} = -24°$ ($c = 1$). – UV.: 265 (3.96), (266 (3.98) [12]). – IR.: 3428, 3220, 3057, 2985, 2930, 1690, 1670 sh., 1666 sh., 1080, 1067. – NMR.: 9.49 br. (*s*, H–N(3)); 7.20 (*d*×*d*, $J = 1.2$, H–C(6)); 5.58 (*d*, $J = 2.5$, H–C(1′)); 5.16–4.86 (*m*, H–C(2′) and H–C(3′)); 4.26 (*d*×*d*, $J = 6.0$ and 2.5, H–C(4′)); 3.85 (*d*, $J = 2.0$, 2 H–C(5′)); 3.26 br. (*s*, HO–C(5′)); 1.98 (*d*, $J = 1.2$, H_3C–C(5)); 1.56 and 1.32 (2*s*, each 3 H, 2 H_3C–C).

1-(5-O-Acetyl-2,3-dideoxy-β-D-glyceropent-2-enofuranosyl)thymine (**5**). To a solution of **1** (130 mg, 0.58 mmol) in anhydrous pyridine (3 ml), acetic anhydride (0.59 ml, 6.23 mmol) was added. The mixture was set aside at RT. for 16 h and then evaporated to dryness. The residue crystallized from methanol (87 mg). Additional 30 mg of the product **5** was isolated from the mother liquor by prep. TLC. (CH_2Cl_2/methanol 5:1, recovery with CH_2Cl_2), Rf 0.70. Yield 76%, m.p. 179–181° (CH_2Cl_2/ether/hexane), $[\alpha]_D^{22} = -58.6°$ ($c = 0.7$). – UV.: 267 (3.76). – IR.: 3571, 3135, 3106, 1742, 1695, 1643, – NMR.: 9.56 (*s*, H–N(3)); 7.24 (*d*, $J = 1.4$, H–C(6)); 7.02 (*hpt*, $J = 1.8$ and 1.4, H–C(1′)); 6.29 (*d*×*d*, $J = 6.1$ and 1.8, H–C(2′)); 5.91 (*d*×*qa*, $J = 6.1$, 2.3 and 1.4, H–C(3′)); 5.05 (*m*, H–C(4′)); 4.43 (*d*×*d*, $J = 12.4$ and 3.8, 1 H–C(5′)); 4.22 (*d*×*d*, $J = 12.4$ and 3.1, the second H–C(5′)); 2.10 (*s*, CH_3CO); 1.99 (*d*, $J = 1.4$, H_3C–C(5)).

$C_{11}H_{12}N_2O_5$ (266.25) Calc. C 54.13 H 5.30 N 10.52% Found C 54.00 H 5.64 N 10.50%

2′,3′-O,O-Isopropylidene-5′-O-methanesulfonyl-5-methyluridine (**6**). The *2′,3′-O,O*-isopropylidene-5-methyluridine (**4**; 75 mg, 0.25 mmol) in anhydrous pyridine (0.7 ml) was treated with methanesulfonyl chloride (0.076 ml, 1 mmol) at 0° and kept aside for 16 h. The mixture was evaporated i.V. to a residue. Prep. TLC. (CH_2Cl_2/acetone 5:1), afforded **6** as foamy product (80 mg, 96%), Rf 0.31, $[\alpha]_D^{18} = -3.5°$ ($c = 1$). – UV.: 263 (3.92). – IR.: 3430 br., 3200 br., 3030, 2985, 2930, 1710 sh., 1690, 1655 sh., 1171. – NMR.: 9.06 (*s*, H–N(3)); 7.12 (*d*×*d*, $J = 1.2$, H–C(6)); 5.65 (*d*, $J = 1.8$, H–C(1′)); 5.08 (*d*×*d*, $J = 6.7$ and 1.8, H–C(2′)); 4.88 (*d*×*d*, $J = 2.9$, H–C(3′)); 4.30–4.59 (*m*, H–C(4′) and 2 H–C(5′)); 3.02 (*s*, CH_3SO_2); 1.91 (*d*, $J = 1.2$, H_3C–C(5)); 1.56 and 1.36 (2 *s*, 2 H_3C–C).

$C_{14}H_{20}N_2O_8S$ (376.38) Calc. C 44.67 H 5.36 N 7.44% Found C 44.54 H 5.62 N 7.68%

5′-Deoxy-5′-iodo-2′,3′-O,O-isopropylidene-methyluridine (7). Compound **6** (150 mg, 0.399 mmol) in butan-2-one (5.5 ml) was treated with sodium iodide (162 mg, 1.08 mmol) and heated under reflux for 2 h. The precipitate was filtered off and the filtrate evaporated to dryness. The residue was partitioned between CH_2Cl_2 and 5% $Na_2S_2O_3$-solution. The organic layer was dried over Na_2SO_4 and evaporated to a powdered residue. The prep. TLC. gave the product **7** (140 mg. 86%), Rf 0.56 (CH_2Cl_2/acetone 5:1), m.p. 146–147°, $[\alpha]_D^{20} = -23.5°$ ($c = 1$). – UV.: 262 (4.07). – IR.: 3460 br., 3182, 3040, 2990, 2980, 2934, 1713, 1695 sh., 1679, 1655 sh., 1090, 1072. – NMR.: 9.64 br. (*s*, H–N(3)); 7.17 (*d*×*d*, $J = 1.2$, H–C(6)); 5.65 (*d*, $J = 2.3$, H–C(1′)); 5.08 (*d*×*d*, $J = 6.4$ and 2.3, H–C(2′)); 4.82 (*d*×*d*, $J = 6.4$ and 3.6, H–C(3′)); 4.25 (*t*×*d*, $J = 6.0$ and 3.6, H–C(4′)); 3.53 (*d*×*d*, $J = 13$ and 6.0, H–C(5′)); 3.36 (*d*×*d*, $J = 13$ and 6.3, the second H–C(5′)); 1.92 (*d*, $J = 1.2$, H_3C–C(5)); 1.56 and 1.36 (2*s*, each 3 H, 2 H_3C–C).

$C_{13}H_{17}IN_2O_5$ (408.21) Calc. C 38.25 H 4.20 N 6.86% Found C 38.51 H 4.43 N 6.47%

2',3'-O,O-Isopropylidene-5-methyl-2,5'-anhydrouridine (**8**). To a solution of **7** (250 mg, 0.612 mmol) in anhydrous methanol (98 ml) silver acetate (459 mg, 2.76 mmol) was added and the mixture heated under reflux for 15 min. The precipitate was filtered off and the excess of silver ion was removed from the filtrate by means of hydrogen sulfide following the procedure reported earlier [17]. The product **8** (127 mg, 74%) crystallized from anhydrous ethanol, m.p. 234–242°, Rf 0.45, $[\alpha]_D^{25} = +14°$ ($c = 1$, DMSO). – UV.: 244 (4.06). – IR.: 3055, 3026, 3005, 2992, 2977, 2945, 1656, 1645, 1095, 1054. – NMR.: 7.91 (*d*, $J = 1.0$, H–C(6)); 5.74 (*s*, H–C(1')); 4.99 (br. *s*, H–C(2') and H–C(3')); 4.68 (br. *s*, H–C(4')); 4.54 (*d*×*d*, $J = 12.8$ and 1.5, H–C(5')); 4.11 (*d*, $J = 12.8$, the second H–C(5')); 1.78 (*d*, $J = 1.0$, H_3C–C(5)); 1.43 and 1.29 (2 *s*, 2 CH_3–C).

$C_{13}H_{16}N_2O_5$ (280.28) Calc. C 55.70 H 5.75 N 10.00% Found C 55.95 H 6.20 N 9.97%

5'-Deoxy-2',3'-O,O-isopropylidene-5'-mercapto-5-methyluridine (**9**). Into a solution of **8** (100 mg, 0.357 mmol) in DMF (4 ml) and triethylamine (0.093 ml), hydrogen sulfide was bubbled for 5 h and left aside for 3 days. The mixture was evaporated i.V. to a residue. Prep. TLC. (CH_2Cl_2/methanol 20:1, two developments) gave **9** (87 mg, 85%), Rf 0.39, m.p. 139–142° (ether), $[\alpha]_D^{27} = +50°$ ($c = 1$). – UV.: 264 (3.98). – IR.: 3440 br., 3200, 3060, 2980, 2924, 1729 sh., 1691 br., 1086 br., 1063 sh. – NMR.: 9.65 (*s*, H–N(3)); 7.08 (*s*, H–C(6)); 5.54 (*d*, $J = 1.7$, H–C(1')); 4.90 (*d*×*d*, $J = 6.2$ and 1.7, H–C(2')); 4.84 (*d*×*d*, $J = 6.2$ and 4.0, H–C(3')); 4.36 (*d*×*d*, $J = 6.5$, H–C(4')); 3.10 (*d*, $J = 6.5$, 2 H–C(5')); 1.91 (*s*, CH_3–C(5)); 1.56 and 1.35 (2 *s*, 2 H_3C–C); 1.21 (*t*, $J = 7.1$, HS–C(5')).

$C_{13}H_{18}N_2O_5S$ (314.35) Calc. C 49.67 H 5.77 N 8.91 S 10.20%
Found „ 49.67 „ 5.72 „ 8.82 „ 10.13%

2',3'-O,O-Isopropylidene-5-methyl-isocytidine (**10**). – a) The compound **8** (100 mg, 0.36 mmol) was dissolved in saturated methanolic ammonia (100 ml) at 0° and then kept aside at RT. for 10 days. The mixture was evaporated to dryness. Prep. TLC. (six developments) afforded **8** (20 mg) and the product **10** (60 mg, 71%), Rf 0.21, m.p. 120° (ext.) (acetone/ether/hexane), $[\alpha]_D^{25} = -58.3°$ ($c = 0.84$). – UV.: 235 sh., 259 sh. (3.81, 4.04). – IR.: 3406 br., 3251 br., 1672, 1666, 1646, 1594. – NMR.: 7.00 (*d*, $J = 1.2$, H–C(6)); 6.57 (*s*, H_2N–C(2)); 5.21–4.95 (*m*, H–C(1'), H–C(2'), and H–C(3')); 4.32 (br. *s*, H–C(4')); 3.95 (br. *s*, 2 H–C(5')); 1.87 (*s*, 3 H_3C–C(5)); 1.58 and 1.35 (2 *s*, 2 H_3C–C).

$C_{13}H_{19}N_3O_5 \cdot (CH_3)_2CO$ (355.38) Calc. C 54.07 H 7.09% Found C 53.93 H 7.38%

b) A cooled solution of 2-*O*-ethyl-2',3'-*O,O*-isopropylidene-5-methyluridine (**14**, 40 mg, 0.123 mmol) in saturated methanolic ammonia (1 ml) was kept aside for 14 days and worked up as described under a). It afforded **10** (25 mg, 78%), identical (Rf, IR., and NMR. spectra) with the product obtained under a).

2-N-Benzoyl-2',3'-O,O-isopropylidene-5-methyl-isocytidine (**11**). The isocytidine **10** (14 mg, 0.047 mmol) was treated with benzoic anhydride (27 mg, 0.12 mmol) in anhydrous pyridine (0.2 ml). The mixture was left at RT. for 4 h and then evaporated i.V. to a residue. Prep. TLC. (CH_2Cl_2/methanol 20:1) separated **11** at Rf 0.26 (11 mg, 61%) and 2-*N*,5'-*O*-dibenzoyl-2',3'-*O,O*-isopropylidene-5-methyl-isocytidine (**12**) at Rf 0.84 (6 mg, 21%).

Data of **11**. M.p. 187–188° (CH_2Cl_2/ether/hexane). – UV.: 250, 274 sh., 301 (4.15, 4.22, 4.36). – IR.: 3402 br., 3070, 2990, 2937, 1692, 1674, 1649, 1592, 1572, 1557 sh., 1100, 1080, 709. – NMR.: 8.28–8.12 and 7.78–7.32 (2 *m*, 2 H and 3 H, 5 arom. H); 7.87 (*d*, $J = 1.2$, H–C(6)); 6.70 (*d*, $J = 1.7$, H–C(1')); 4.89 (*m*, H–C(2') and H–C(3')); 4.44 (*d*×*d*, $J = 2.7$, H–C(4')); 4.09 (*d*×*d*, $J = 2.4$ and 11.7, H–C(5')); 3.91 (*d*×*d*, $J = 11.7$ and 2.7, the second H–C(5')); 3.09 (br. *s*, HO); 1.95 (*d*, $J = 1.2$, H_3C–C(5)); 1.70 and 1.39 (2 *s*, 2 H_3C–C). – MS. (*m/z*): 401 (M^+).

$C_{20}H_{23}N_3O_6$ (401.40) Calc. N 10.47% Found N 10.52%

Data of **12**. M.p. 63–64° (CH_2Cl_2/ether/hexane). – UV.: 232, 252, sh., 276 sh., 284 sh., 301 (4.26, 4.14, 4.22, 4.34). – IR.: 3448 br., 3066, 2986, 2924, 1724, 1685 br., 1646, 1600, 1574, 1082 br., 709. – NMR.: 8.45–7.34 (*m*, 1 + 10 H, H–C(6) and 10 arom. H); 6.47 (*d*, $J = 2.2$, H–C(1')); 5.01 (*d*×*d*, $J = 2.2$ and 6.0, H–C(2')); 4.89 (*d*×*d*, $J = 6.0$ and 2.9, H–C(3')); 4.69 (*d*×*d*, $J = 2.93$ and 11.7, H–C(5')); 4.53 (*d*×*d*, $J = 11.7$ and 2.6, the second H–C(5')); 1.73 and 1.42 (2 *s*, 2 H_3C–C); 1.64 (*d*, $J = 1.1$, H_3C–C(5)). – MS. (*m/z*): 505 (M^+).

Treatment of 5'-iodo-5-methyluridine (**7**) *with ethanolic potassium hydroxide.* A suspension of **7** (40 mg, 0.1 mmol) in anhydrous ethanol (10 ml) was treated with ethanolic 0.5M KOH (0.4 ml, 0.2 mmol), kept aside at RT. for 16 h, and then at 40° for 4 h. The mixture was evaporated i.V. to dryness and the residue dissolved in methanol. Prep. TLC. separated 2-*O*-ethyl-2',3'-*O*,*O*-isopropylidene-5-methyluridine (**14**) at Rf 0.34 (14 mg, 50%) and 1-(5-deoxy-2,3-isopropylidene-β-D-erythropent-4-enofuranosyl)5-methyluracil (**13**) at Rf 0.53 (12 mg, 43%).

Data of **14**. M.p. 202–204° (anhydrous ethanol), $[\alpha]_D^{23} = -52.5°$ ($c = 1$, DMSO). – UV.: 233 sh., 254 (3.92, 4.03). – IR.: 3525, 3305, 1662, 1621. – NMR.: 7.72 (*s*, H–C(6)); 5.82 (*d*, $J = 2.4$, H–C(1')); 5.18 (*t*, $J = 4.9$, HO); 4.91 (*d*×*d*, $J = 2.4$ and 5.9, H–C(2')); 4.75 (*d*×*d*, $J = 5.9$ and 3.0, H–C(3')); 4.35 (*qa*, $J = 7.3$, CH_2CH_3); 4.11 (*d*×*d*, $J = 3.9$, H–C(4')); 3.59 (*m*, 2 H–C(5')); 1.78 (*s*, H_3C–C(5)); 1.49 and 1.29 (2 *s*, 2 H_3C–C); 1.31 (*t*, $J = 7.3$, CH_3CH_2). – MS. *(m/z):* 326 (M^+).

$C_{15}H_{22}N_2O_6$ (326.35) Calc. C 55.20 H 6.80 N 8.58% Found C 55.14 H 7.10 N 8.23%

Data of **13**. M.p. 74–75° (CH_2Cl_2/ether/hexane), $[\alpha]_D^{23} = +60°$ ($c = 1$). – UV.: 264 (3.84). – IR.: 3486 br., 3206, 1724 sh., 1692, 1619. – NMR.: 9.30 (br. *s*, H–N(3)); 7.00 (*s*, H–C(6)); 5.61 (*s*, H–C(1')); 5.33 (*d*, $J = 5.9$, H–C(2')); 5.04 (*d*, $J = 5.9$, H–C(3')); 4.56 (*d*, $J = 1.8$, H–C(5')); 4.38 (*d*, $J = 1.8$, the second H–C(5')); 1.92 (*s*, H_3C–C(5)); 1.51 and 1.38 (2 *s*, 2 H_3C–C).

$C_{13}H_{16}N_2O_5$ (280.28) Calc. C 55.70 H 5.75 N 10.00% Found C 55.81 H 5.94 N 10.02%

2-O-Ethyl-2',3'-O,O-isopropylidene-5-methyluridine (**14**). To a suspension of **8** (56 mg, 0.2 mmol) in anhydrous ethanol (20 ml) ethanolic 0.5M KOH (0.4 ml, 0.2 mmol) was added. The mixture was stirred at RT. for 30 min. and then evaporated to dryness i.V. Prep. TLC. (CH_2Cl_2/CH_3OH 20:1, three developments) separated **8** (14 mg) and the product **14** (37 mg, 75%), identical (m.p., IR. and NMR. spectra) with the product obtained from the 5'-iodo-compound 7.

1-(5-Deoxy-2,3-O,O-isopropylidene-α-L-lyxopentofuranosyl)5-methyluracil (**15**). To a solution of 4',5'-unsaturated compound (**13**, 42 mg, 0.15 mmol) in ethanol (8 ml) 5% Pd/C (26 mg) was added and shaken in an atmosphere of hydrogen for 15 h. The catalyst was filtered off and the filtrate evaporated to dryness. The chromatographically pure residue (42 mg, 99%), Rf 0.30 (CH_2Cl_2/CH_3OH 20:1) crystallized from acetone, m.p. 178–179°, $[\alpha]_D^{24} = -19°$ ($c = 1$). – UV.: 266 (3.92). – IR.: 3503 br., 3214, 3058, 2983, 2815, 1721, 1711, 1696, 1075. – NMR.: 9.24 (br. *s*, H–N(3)); 7.05 (*d*, $J = 1.1$, H–C(6)); 5.31 (br. *s*, H–C(1')); 5.26 (*d*, $J = 5.9$, H–C(2')); 4.85 (*d*×*d*, $J = 3.8$ and 5.9, H–C(3')); 4.57 (*d*×*qa*, $J = 3.8$ and 6.3, H–C(4')); 1.92 (*d*, $J = 1.1$, H_3C–C(5)); 1.52 and 1.36 (2 *s*, each 3 H, 2 H_3C–C); 1.33 (*d*, $J = 6.3$, H_3C–C(4')).

$C_{13}H_{18}N_2O_5$ (282.29) Calc. C 55.31 H 6.43 N 9.93% Found C 55.25 H 6.54 N 10.05%

5'-Deoxy-2',3'-O,O-isopropylidene-5-methyluridine (**16**). To a solution of 5'-iodo compound (**7**, 81.6 mg, 0.2 mmol) in ethanol (10 ml) 5% Pd/C (53 mg) and 1N NaOH (0.4 ml) were added and then stirred in hydrogen atmosphere for 4 h. The solution was filtered and evaporated to dryness i.V. and then partitioned between chloroform and water. The chloroform layer was washed with a solution of $Na_2S_2O_3$, $NaHCO_3$ and water, and then evaporated i.V. to a residue. Prep. TLC. (CH_2Cl_2/CH_3OH 20:1, two developments) afforded **16** as foamy product (40 mg, 71%), Rf 0.31, $[\alpha]_D^{22} = -22.5°$ ($c = 1$). – UV.: 264 (3.90). – IR.: 3523 br., 3203, 3058, 2983, 2933, 1720, 1707, 1691, 1086, 1069. – NMR.: 9.10 (br. *s*, H–N(3)); 7.06 (*d*, $J = 1.1$, H–C(6)); 5.63 (*d*, $J = 2.3$, H–C(1')); 4.96 (*d*×*d*, $J = 2.3$ and 6.6, H–C(2')); 4.52 (*d*×*d*, $J = 4.7$ and 6.6, H–C(3')); 4.17 (*d*×*d*, $J = 6.7$ and 4.7, H–C(4')); 1.93 (*d*, $J = 1.1$, H_3C–C(5)); 1.56 and 1.34 (2 *s*, 2 H_3C–C); 1.40 (*d*, $J = 6.7$, H_3C–C(4')).

$C_{13}H_{18}N_2O_5$ (282.29) Calc. C 55.31 H 6.43 N 9.93% Found C 55.32 H 6.69 N 10.16%

5-Methyl-5,6-dihydrouridine (**17**). To a solution of 5-methyluridine **2** (160 mg, 0.62 mmol) in water (29 ml), 5% Rh/Al_2O_3 (50 mg) was added. The mixture was shaken in an atmosphere of hydrogen (0.34 MPa) at RT. for 8 h. The catalyst was filtered off and the filtrate evaporated to dryness. Prep. TLC. (CH_2Cl_2/CH_3OH 5:1) yielded a mixture of stereoisomeric products (132 mg, 83%), Rf 0.05, $[\alpha]_D^{29} = -32°$ ($c = 1$, CH_3OH). After several recrystallizations (methanol/ether), a pure isomer (38 mg) was isolated from mother liquor fraction, m.p. 151–153°, $[\alpha]_D^{26} = +25.9°$ ($c = 0.85$, CH_3OH). – NMR.: 10.23 (*s*, H–N(3)); 5.67 (*d*, $J = 5.6$, H–C(1')); 5.09 (*d*, $J = 5.4$, HO–C(2')); 4.95 (*d*, $J = 4.6$, HO–C(3')); 4.80 (*t*,

J=5.4, HO–C(5')); 4.07–3.79 (*m*, H–C(2') and H–C(3')); 3.66 (*m*, H–C(4')); 3.48 (*d*, J=4.0, 2 H–C(5')); 3.09 (*d*×*d*, J=12.4, H–C(6)); the second H–C(6) is obscured by the signal of H_2O; 1.07 (*d*, J=6.7, H_3C–C(5)).

2',3'-O,O-Isopropylidene-5-methyl-5, 6-dihydrouridine (**18**). To a solution of **4** (160 mg, 0.54 mmol) in water (25 ml), 5% Rh/Al_2O_3 (42.9 mg) was added, hydrogenated, and worked up as described for compound **17**. Prep. TLC. (CH_2Cl_2/CH_3OH 15:1) gave a foamy mixture of stereoisomers (125 mg, 78%), Rf 0.35. – IR.: 3470 br., 3240 br., 1721, 1700 br. – NMR.: *(Isomer A):* 5.46 (*d*, J=2.9, 0.6 H, H–C(1')); 1.27 (*d*, J=6.8, 1.8 H, H_3C–C(5)); *(Isomer B):* 5.32 (*d*, J=3.4, H–C(1')); 1.25 (*d*, J=6.8, 1.2 H, H_3C–C(5)).

$C_{13}H_{20}N_2O_6$ (300.31) Calc. C 51.99 H 6.71 N 9.33% Found C 51.61 H 6.82 N 8.96%

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