Larger laboratory scale synthesis of 5-methyluridine and formal synthesis of its L-enantiomer

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Abstract

A larger laboratory scale synthesis (>60 g per run) of 5-methyluridine is presented. The critical intermediate 1,2-O-isopropylidene-α-D-ribofuranose was prepared from very cheap D-glucose via D-allose. Its L-enantiomer was obtained from L-arabinose via L-glucose, and also from L-xylose.

Keywords: 5-Methyluridine, chiral pool, stereoselective synthesis, D- and L-carbohydrates
Introduction

5-Methyluridine 1 is a component of the ribonucleic acids from which it can be isolated, however demand for it far exceeds a supply in this way. 5-Methyluridine is a starting compound for the synthesis of 3'-azido-2',3'-dideoxythymidine or AZT (Zidovudine, Retrovir) 3 (via 2'-deoxy compound 2) and also to obtain 2',3'-dideoxy-2',3'-didehydrothymidine or d4T 4, both used as potent inhibitors of the reverse transcriptase, a critical enzyme necessary for multiplication of the HIV virus responsible for the AIDS epidemic.\(^1\) The sugar moieties present in 1-4 belong to the D series. L-Thymidine 6 is very active inhibitor of the reverse transcriptase of the hepatitis B virus (HBV).\(^2\) Compound 6 is marketed under the name Telbivudine (Sebvio, Tyzeka) and is the enantiomer of 2. As such it can be prepared from the L 5-methyluridine 5 via deoxygenation at its 2’-position.\(^3\) Considering the demand for both enantiomers 1 and 5, uniform access to both of them is an attractive synthetic goal.

\[
\begin{align*}
1 & \quad \quad 2 \quad \quad 3 \quad \quad \text{AZT} \\
4 & \quad \quad 5 \quad \quad \text{5-methyluridine} \\
6 & \quad \quad \text{Telbivudine}
\end{align*}
\]

The stereochemical characteristics of 1,2;5,6-di-O-isopropylidene-α-D-glucofuranose 7 permit its transformation into 1,2-O-isopropylidene-α-D-ribofuranose 12\(^4\) and further to 1-O-acetyl-2,3,5-tri-O-benzyl-β-D-ribofuranose 15 and finally to 5-methyluridine 1. It is obvious that the same sequence of reactions performed on L-glucose will furnish L 5-methyluridine 5. Compound 15 can be directly prepared form D-ribose using HCl-MeOH (→ 13),\(^5,6\) H\textsubscript{2}SO\textsubscript{4}-MeOH\(^7\) or AcCl-MeOH\(^8\) followed by benzoylation (→ 14) and acetylation (→ 15), but since D-glucose is abundant and is one of the cheapest chiral compounds available, we used it to obtain D-ribose and finally 1 at a larger laboratory scale (> 60 g per run) in excellent cumulative yields of ca. 90% for the sequence 15→ 17→ 1. We also tried to obtain 1,2-O-isopropylidene-α-L-ribofuranose 32 using L-glucose which was obtained from L-arabinose via the nitromethane one-carbon extension (Fischer-Sowden reaction\(^9,10\)) which is a carbohydrate version of the Henry reaction,\(^11-15\) followed by a Nef reaction (sodium acetate, H\textsubscript{2}SO\textsubscript{4}).\(^10\) The other versions of this process include ozonization,\(^16,17\) and peroxomolybdate-H\textsubscript{2}O\textsubscript{2};\(^17,18\) non-carbohydrate examples include among others “basic silica gel”,\(^19\) Na\textsubscript{2}CO\textsubscript{3}\(^20\) and H\textsubscript{2}O\textsubscript{2}-K\textsubscript{2}CO\textsubscript{3};\(^21\) the Nef reaction has been recently reviewed.\(^22,23\) This met with a limited success due to difficulties in separation of the necessary L-glucose 27 from the L-mannose 28 by chromatography (see below). A much better result was obtained using reasonably priced L-xylose which was converted to the L-ribose 32 in batches of ca. 15 g per run. Since 32 can be elaborated to produce L 5-methyluridine 5 by the same set of conditions as those applied to reach 5-methyluridine 1 from the D-ribose 12, we can claim that a formal synthesis of L 5 was also realized.
Results and Discussion

A transformation of D-glucose into D-ribose via inversion of the configuration at the C3 position followed by fission of the C5–C6 bond (dehomologation) is known, and we followed the published procedures with some additional modifications. Alternative methods to produce D-ribose have been reviewed. The critical inversion of configuration at the C3 position of D-glucose to get D-allose \(9\) requires the transient ulose \(8\). Reduction of \(8\) proceeds with practically complete stereoselectivity \((\rightarrow 9)\) using NaBH\(_4\) but application of the LiAlH\(_4\) gives mixtures of \(7\) and \(9\).\(^{25}\) Some care must be exercised during the oxidation step \((7 \rightarrow 8)\). The cheapest and operationally easiest oxidant is DMSO-Ac\(_2\)O mixture,\(^{26}\) even though foul smelling divalent sulfur compounds are formed. The other oxidants include DMSO-P\(_2\)O\(_5\),\(^{27}\) pyridinium dichromate-Ac\(_2\)O,\(^{28}\) pyridinium chlorochromate,\(^{29}\) and RuCl\(_3\)-NaIO\(_4\),\(^{30}\) (RuO\(_2\)-NaIO\(_4\),\(^{31}\) was reported to also form Baeyer-Villiger overoxidation products) and finally Dess-Martin periodinane.\(^{32}\) DMSO-Ac\(_2\)O is not a very active system and its application may result in incomplete conversion of \(7\) into \(8\), and consequently, the next reduction step may furnish a mixture of the allose \(9\) and unreacted glucose \(7\), which are difficult to separate. In fact, unreacted \(7\) was reported to be present even after 24 h of reaction time.\(^{26}\) The same kind of problem has been observed before in different context.\(^{33}\) The very active CrO\(_3\)-Py-Ac\(_2\)O mixture\(^{34}\) provided complete oxidation in less than 1 hour at room temperature for 25-39 g batches of \(7\). A weak point of this method is formation of insoluble tars composed of the reduced chromium compounds complexed with pyridine. A very interesting one-pot oxidation-reduction procedure was published which consists of the addition of the NaBH\(_4\) directly to the DMSO-oxalyl chloride oxidation mixture,\(^{35}\) although this was performed on small scale. The dehomologation step \((9 \rightarrow 10 \rightarrow 11 \rightarrow 12)\), i.e. a transformation of the D-allose (a hexose) to D-ribose (a pentose) can be performed using orthoperiodic acid H\(_5\)IO\(_6\) followed by NaBH\(_4\) reduction.\(^{36,37}\) The orthoperiodic acid is strong enough (pK\(_a\) ca 3.3\(^{38}\)) to promote a hydrolysis of the more reactive C5–C6 acetonide in \(9\) to liberate a diol \(10\) which was subsequently cleaved by the same reagent to furnish the aldehyde \(11\) which in turn was subjected to NaBH\(_4\) reduction to yield the ribo compound \(12\). This procedure is very attractive and in fact it was successfully used on a small scale (1 g) in good yields (e.g. \(29 \rightarrow 32\), see below). When applied to \(9\) at a 27 g scale however, it furnished the 1,2-O-isopropylidene-α-D-ribofuranose \(12\) in yield as low as 25%. The reason for this is unclear, but probably can be traced to incomplete removal of the iodic acid HIO\(_3\) formed during oxidation of the C5–C6 diol. HIO\(_3\) crystallized in the reaction mixture and must have been filtered off. In the case of incomplete crystallization and removal, NaBH\(_4\) reduced it to the transient hypiodous acid\(^{39}\) which presumably degraded the aldehyde \(11\) oxidatively. To avoid this it was much better to perform separately the hydrolysis \((9 \rightarrow 10)\) using 0.8% H\(_2\)SO\(_4\) in MeOH-H\(_2\)O mixture\(^{40}\) followed by NaIO\(_4\) oxidation \((\rightarrow 11)\) and final NaBH\(_4\) reduction to get the 1,2-O-isopropylidene-α-D-ribofuranose \(12\) in much better yield \((9 \rightarrow 12, 76\%)\). Removal of the acetonide (70% aq. AcOH, 80 °C) followed by Fischer type glycosylation (MeOH, cat. H\(_2\)SO\(_4\) or HCl) furnished kinetically controlled furanosides \(13\) which, upon conventional benzoylation (BzCl, Py) followed by acetylation (Ac\(_2\)O, AcOH, cat. H\(_2\)SO\(_4\)), furnished the necessary 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose \(15\). The best cumulative yields for this sequence was 45% based on \(12\). There are some variations in the published conditions and the yields of \(15\) obtained by acetylation.\(^{5,6,41}\) Cimpoia \(et\ al.\)^{41} reported that the best procedure was with a decreased amount of H\(_2\)SO\(_4\) in relation to AcOH and Ac\(_2\)O and low temperature to avoid formation of the open-chain acetal acetates. Transformations of \(12\) to \(15\) were performed without isolation of the intermediates. The compound \(15\) must be thoroughly dried since traces of \(i\)-PrOH used for its crystallization react with SnCl\(_4\) during the next coupling step to liberate HCl which may compromise the yield of \(17\). The compound \(15\) was finally coupled with the trimethysilylated thymine (thymine, HMDS, cat.(NH\(_4\))\(_2\)SO\(_4\), bp., 3-4 h) under the influence of SnCl\(_4\) in CH\(_3\)CN\(^{42}\) to get 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl.
thymine 17 in nearly quantitative yield. Alternative versions of this coupling include different activating groups at the anomeric position, like chloride,\textsuperscript{43} methylcarbonate,\textsuperscript{44} 1,2-epoxide,\textsuperscript{45} N-phenyl trifluoracetimidate,\textsuperscript{46} or S-tolyl group,\textsuperscript{47} different catalysts like TMSOTf,\textsuperscript{48} BiBr\textsubscript{3},\textsuperscript{49} or Ph\textsubscript{3}PAu\textsuperscript{+} \(\text{N(SO}_{2}CF_{3})_{2}\),\textsuperscript{50} different pattern of protection of the ribofuranosyl moiety like 1,2,3,5-tetra-O-acetate\textsuperscript{49} or 1,2-di-O-acetyl-3,5-di-O-benzoate,\textsuperscript{51} different activation of the thymine moiety via bis(trimethylsilylation)\textsuperscript{52} rather than bis(trimethylsilylation), and finally a solvent-free ball milling procedure.\textsuperscript{53} Compound 17 was finally deprotected using Zemplén conditions (MeOH, cat. NaOMe). Methyl benzoate (an oil) formed during this reaction interferes with the crystallization of 1 and for this reason was removed by partition between CHCl\textsubscript{3} and water (1 remained in the water phase). The 5-methyluridine 1 formed in this way was isolated in ca 90\% yield for two steps (coupling and deprotection). It is interesting to note that the commercial 1,2,3,5-tetra-O-acetyl-\(\beta\)-d-ribofuranose 16 under the same coupling conditions furnished 2,3,5-tri-O-acetyl-\(\beta\)-d-ribofuranosyl thymine 18 in lower yield (69.5\%) even though a clear spot-to-spot reaction also took place. This probably can be attributed to inferior stabilization of the reactive cation 20 in comparison to the benzylic cation 19. Scheme 1 summarizes this part of the project.

`Scheme 1. Synthesis of 5-methyluridine 1 starting from d-glucose via d-allose.`
L-Ribose\(^{54,55}\) a necessary substrate to obtain the L 5-methyluridine 5 is a known compound that can be obtained from L-arabinose via epimerization at the C2 position catalyzed by molybdic acid (Bilik reaction)\(^{56,57}\) starting from D-fructose\(^{58}\) D-galactose\(^{59}\) D-lyxose\(^{60}\) or D-ribose via transposition of the C1–C5 position.\(^{61,62}\) The latter transformation is possible due to the enantiotropic relationship between both –CH\(_2\)OH groups in D-ribitol (and also in any alditol which has a plane of symmetry like galactitol or allitol. In fact D-galactose was transformed into L-galactose\(^{63}\) by the same kind of transposition). However, having accomplished a synthesis of 1 using D-glucose, we wanted to apply L-glucose for the same purpose to obtain the L enantiomer 5. L-Glucose can be prepared from D-glucose by the published procedure.\(^{64}\) It is also commercially available but due to the prohibitively high price we tried to obtain it from reasonably priced L-arabinose via a one carbon atom extension. This approach is shown in the Scheme 2. The Henry reaction (aldehyde/ketone, nitroalkane, base)\(^{9-15}\) performed on L-arabinose and CH\(_3\)NO\(_2\) and NaOMe\(^{9,10}\) furnished a mixture of crystalline 1-deoxy-1-nitro-L-glucitol 21 and 1-deoxy-1-nitro-L-mannitol 22 in approximately equal proportion. This addition to diastereotopic sides of the carbonyl group should follow the Felkin-Anh model\(^{65}\) as shown in 23 and 24 (Figure 1) where the C3-C5 fragment is treated as a large group and the C2-OH as a medium one.

\[ \text{L-Arabinose} \xrightarrow{\text{CHO}} \text{L-Glucose 25} \]

\[ \text{L-Manose 26} \]

\[ \text{27} \text{ more polar, acetonide of L-glucose} \]

\[ \text{28} \text{ less polar acetonide of L-mannose} \]

\[ \text{R':} \quad \text{R:} \]

\[ \text{L-Allose 29} \]

\[ \text{L-Allose 30} \]

\[ \text{L-Ribose 32} \]

Conditions: a. CH\(_3\)NO\(_2\), NaOMe; b. H\(_2\)SO\(_4\), H\(_2\)O; c. acetone, H\(^+\), ultrasound; d. CrO\(_3\), Py, Ac\(_2\)O; e. NaBH\(_4\); f. H\(_5\)IO\(_6\) 29 via 30 to 31, one pot.

Scheme 2. Synthesis of 1,2-O-isopropylidene-α-L-ribose 32 starting from L-arabinose.
Figure 1. Stereochemistry of formation of nitroalcohols 21 and 22 according to the Felkin-Anh model (A) and according to the ref. 66 (B).

The privileged attack on the Re side should furnish the R configuration at the C2 atom, i.e. the L-gluco diastereoisomer 21 should predominate. It is not a case, though. A reason for this is unclear, but one can consider extensive hydrogen bonding between a solvent (MeOH) and the C=O group. This may override the steric and stereoelectronic factors which control the Felkin-Anh transition state. Also, a fully formed carbonyl group might not even have been present. Rather, reactive forms might have been the species with the hemiacetal rings being partially opened with retained α or β configuration. This may additionally influence the steric outcome. Theoretical calculations show that the transition states during the Henry reactions are such, that the negatively charged NO₂ moiety is far away from the carbonyl oxygen atom as in 23a and 24a which apparently have the same energies. Consequently, both epimeric products 21 and 22 are formed without much stereoselection. This is additionally influenced by reversibility of the addition in basic medium. Irrespective of the mechanism, there is no preference for 21 over 22. The 21/22 mixture was described as being separable by tedious fractional crystallization⁹,¹⁰ and the resulting 1-nitro-L-glucitol and 1-nitro-L-mannitol were subjected separately to the Nef reaction to give L-glucose and L-mannose, respectively. However, since we needed the 2,3;5,6-di-O-isopropylidene-L-mannofuranose for another project we performed the Nef reaction (a. 21/22, NaOH; b. H₂SO₄)¹⁰ without fractional crystallization. A mixture of L-glucose 25 and L-mannose 26 thus obtained was subjected to isopropyldenation (acetone, H₂SO₄, ultrasound)⁶⁷ to yield a mixture of the di-O-isopropylidened compounds 27 and 28 which were separated by vacuum-dry chromatography⁶⁸,⁶⁹ at this stage. Eluted first was the L-mannose 28 followed by more polar L-glucose 27. The NMR characteristics of both 27 and 28 are identical to those of their D-enantiomers. Due to small difference of the Rᵣ values of 27 and 28, their separation was successful at small scale only (ca 2 g of mixture per run). The 1,2;5,6-di-O-isopropylidene-α-L-glucofuranose 27 was then subjected to the oxidation/reduction (a. CrO₃-Py-AC₂O;₃⁴ b. NaBH₄) sequence to invert the configuration at the C3 position to produce the L-allose 29 which in turn was subjected to dehomologation (a. H₃IO₆, b. NaBH₄³⁶,³⁷) at a 1 g scale which furnished the 1,2-O-isopropylidene-α-L-ribofuranose 32 in a cumulative 69% yield (29→32). As mentioned above for the D-enantiomer this process did not function well at elevated scale. Considering the overall length of this process, difficulties during the separation of 27 and 28, and the low overall yield of the 1,2-O-isopropylidene-α-L-ribofuranose 32, a more efficient route was devised using L-xylene.
### Scheme 3. Alternative access to L-ribose 32 via L-xylene.

This commercially available pentose was transformed in one pot\(^70\) [a. acetone, H\(_2\)SO\(_4\); b. partial neutralization with Na\(_2\)CO\(_3\), H\(_2\)O] to the 1,2-O-isopropylidene-\(\alpha\)-L-xylene. Selective protection of the primary OH group via silylation (t-BuMe\(_2\)SiCl, imidazole; t-BuPh\(_2\)Si\(^-\)71\) and Tr\(^-\)72 were published for the same purpose) was nearly quantitative (34 → 35). The compound 35 was subjected to inversion of configuration at the C3 position via oxidation-reduction (a. CrO\(_3\)-PyAc\(_2\)O; \(^34\) b. NaBH\(_4\)) followed by desilylation to furnish the 1,2-O-isopropylidene-\(\alpha\)-L-ribofuranose 32 with a total selectivity and good yield. Compound 32 is the \(\alpha\) enantiomer of the intermediate \(\alpha\) 12, and can be further elaborated to obtain the \(\alpha\) 5-methyluridine 5.

### Conclusions

In conclusion, a larger laboratory scale route to obtain 5-methyluridine 1 is described using \(L\)-glucose as a precursor of the pivotal 1,2-O-isopropylidene-\(\alpha\)-\(D\)-ribofuranose 12. Its enantiomer \(L\) 32 was obtained starting from either \(L\)-arabinose via \(L\)-glucose and \(L\)-allole, or (much better) from \(L\)-xylene.

### Experimental Section

**General.** EtOAc was dried by azeotropic removal of water; ca. 20% of a forerun was rejected and the rest was distilled. Acetone and CH\(_2\)Cl\(_2\) were dried by shaking with P\(_2\)O\(_5\) during 20 min, rapid filtration and distillation. DMF was dried by azeotropic removal of water using benzene or toluene (ca. 20% of the volume of DMF). Pyridine was dried by storage over KOH. MeOH was dried by Mg/I\(_2\) method. The \(^1\)H and \(^13\)C spectra were obtained on the Varian 300 MHz spectrometer unless otherwise stated. Exact mass measurements were obtained on the Waters Xevo G2-XS QTof spectrometer. Optical rotations were measured on the Jasco P-2000 241 automatic polarimeter at ca 26 °C. Moisture-sensitive reactions were performed using protecting atmosphere of argon dried by passage through “blue silica gel”. Evaporations of the solvents were performed at ca 40 °C. MgSO\(_4\) was used to dry the extracts. Column chromatography was performed using silica gel 70-
230 mesh from the Fluka. TLC chromatography was performed on the 0.2 mm silica gel aluminum plates (Fluka) and the spots were revealed using 10% H₂SO₄ in MeOH and heating at ca. 110 °C.

1,2;5,6-Di-O-isopropylidene-α-d-allofuranose 9. A round-bottom flask equipped with a reflux condenser and magnetic stirring bar was charged with CH₂Cl₂ 700 (mL) and CrO₃ (60 g, 600 mmol). The flask was immersed in ice-water, and pyridine (97 mL, 1200 mmol) was added portion wise during 10 min. The cooling bath was removed. After stirring for 1 h at rt, the dark brown mixture was cooled again with ice-water bath and 7 (39.3 g, 151 mmol) was added portionwise. After each addition of 7 a small volume of AC₂O was added. The total volume of AC₂O was 75 mL, 61.3 g, 600 mmol. After these intermittent additions which required 15 min the cooling bath was removed and oxidation continued for 25 min counting from the end of additions. TLC showed complete conversion of 7 Rf 0.49 into more polar ulose 8 Rf 0.33 (hexane - EtOAc 1:1). Most of CH₂Cl₂ was evaporated below 40 °C and 1:1 mixture of toluene - EtOAc, (500 mL) was added. This resulted in precipitation of insoluble black tar. The supernatant was decanted and the solid residue was washed twice with the same solvent. The combined solutions were passed through a short silica gel column prepared in toluene - EtOAc 1:2 using over pressure. The column was eluted with the same solvent system and product-containing fractions were evaporated. Xylenes (100 mL) were added and evaporation was continued to expel all residual pyridine. The oil obtained was dissolved in 96% EtOH (300 mL), cooled in ice-water bath and NaBH₄ (5 g, 132 mmol) was added portionwise while maintaining magnetic stirring. The cooling bath was removed and stirring was continued overnight. TLC showed allose 9 Rf 0.36 slightly less polar than the ulose 8 (hexane - EtOAc 1:1). Acetone (10 mL) was added to destroy the excess of NaBH₄ and most of the volatiles were evaporated. The residue was taken up in CH₂Cl₂ and washed with water. The organic phase was dried (MgSO₄) and evaporated to yield solid crude 9 (31.1 g, 79% for two steps). Mp 72-75 °C (hexane - EtOH), [α]D 26.7 (c 2 CHCl₃); lit. 32 mp 74-75 °C (toluene), [α]β +38.8 (c 1.03 CHCl₃). NMR: see L-enantiomer 29.

1,2-O-Isopropylidene-α-d-ribofuranose 12

A. Via H₂IO₆/NaBH₄. Compound 9 (27 g, 103.8 mmol) was dissolved in dry EtOAc (150 mL) and stirred with H₂IO₆ (99% pure, 28.7 g, 124.6 mmol) added in three portions. A white precipitate soon appeared. TLC showed the aldehyde 11 Rf 0.29 (hexane - EtOAc 1:2); the substrate 9 has Rf 0.36 (hexane - EtOAc 1:1). After 2 h filtration was performed using a sintered glass and the precipitate was washed with EtOAc. The volatiles were removed by evaporation. Some solid material appeared. EtOAc (50 mL) was added and filtration/evaporation was repeated. The residual oil was briefly dried on an oil pump, dissolved in 96% EtOH (100 mL), cooled in ice-water bath and treated with NaBH₄ (5 g, 132 mmol). The cooling bath was removed. Rf of the product 12 was 0.46 in CH₂Cl₂ - MeOH 20:1.5. After 3 h most of EtOH was evaporated and the residue was dissolved in CH₂Cl₂ and this solution was washed with water. The water phase was back extracted twice with CH₂Cl₂. The combined organic phases were dried, filtered and evaporated. Purification by chromatography CH₂Cl₂ - MeOH 20:1.4 furnished 12 (4.9 g, 25%).

B. Via stepwise hydrolysis and NaIO₄/NaBH₄ treatment. To a cold (ice bath) solution of 9 (35 g, 134.6 mmol) in MeOH (300 mL) was added cold 0.8% H₂SO₄ (250 mL), and the mixture was left overnight at rt. TLC showed that 9 (Rf 0.36 in hexane - EtOAc 1:1) reacted to form 10 Rf 0.0 in the same solvent system, or Rf 0.21 in CH₂Cl₂ - MeOH 9:1. Amberlite IRA 410 (OH⁻) was added to neutralize the acid and was removed by filtration and washed with MeOH. The volatiles were removed by evaporation to yield crude 10 as a syrup. A small amount of this material was purified by chromatography using CH₂Cl₂ - MeOH 9:1 to get the crystalline material, mp 130-134 °C (EtOAc - EtOH); lit. 32 mp. 131-133 °C (Et₂O - MeOH) The bulk of the crude product was dissolved in 96% EtOH (250 mL) and treated with a suspension of NaIO₄ (30 g, 140 mmol), in H₂O (100 mL) with magnetic stirring. A white precipitate started to deposit immediately. After 4 h TLC showed a conversion of 10 into the
aldehyde 11 Rf 0.29 in hexane - EtOAc 1:2. The whole mixture was filtered on a sintered glass and the solid material was washed with EtOH. NaBH₄ (6 g, 158.7 mmol) was added to the cold (ice bath) filtrate and the mixture was stirred magnetically during 5 h at rt. The work-up and purification as described above furnished 12 (16.9 g, 76% for three steps). 12: mp 85 °C (hexane - EtOAc), [α]₀^{26} + 69 (c 2.2, EtOH); lit.⁷³ 85.5-86 °C, [α]₀ + 65 (c 1.0 EtOH). NMR: see L enantiomer 32.

1-O-Acetyl 2,3,5-tri-O-benzoyl-β-d-ribofuranose 15. The conversion of 12 into 15 was performed without isolation of the intermediates. The acetonide 12 (25 g, 132 mmol) in 70% AcOH (150 mL) was maintained at 80 °C during 3 h, whereupon the volatiles were removed by evaporation below 40 °C. Coevaporation with xylenes and drying on an oil pump furnished glassy material, which was dissolved in dry MeOH (400 mL) and the mixture was cooled down in ice bath and magnetically stirred. H₂SO₄ (97%, 2 mL) was added slowly and the mixture was left for 18 h in a refrigerator. Saturated aq Ba(OH)₂ was added to neutrality and precipitated BaSO₄ was removed by filtration through Celite. The volatiles were removed on an evaporator and on an oil pump. To the residue was added pyridine(300 mL), followed by BzCl (54 mL, 64.6 g, 460 mmol), added dropwise under magnetic stirring and with cooling in an ice bath and under a blanket of argon. After an overnight reaction, TLC (CHCl₃ - MeOH 9:1) showed two products having Rf 0.68 and 0.80, presumably both anomeric compounds 14. Water (5 mL) was added to hydrolyze the excess of BzCl and 2 h later an extraction was performed (CH₂Cl₂ - ice - 5N HCl). The organic phase was washed with aq Na₂CO₃, water (2 x), dried and evaporated. To the residue (58 g) was added AcOH (40 mL) and Ac₂O (90 mL). The solution was chilled in an ice - salt bath and conc. H₂SO₄ (14 mL) was added dropwise under a blanket of argon and with manual swirling. The flask was closed with a rubber septum and left at ca. -5 °C for 10 h. TLC showed the compound 15 Rf 0.60 (hexane – EtOAc, 2:1) together with less and more polar byproducts. CH₂Cl₂ was added and the solution was transferred to a separatory funnel charged with ice and water and extraction was performed. The organic layer was washed with water (3 x), dried and evaporated. Addition of i-PrOH resulted in spontaneous crystallization. Filtration, washing with cold i-PrOH and prolonged drying on an oil pump furnished 15 (29.8 g, 45% cumulative yield), which was the best yield obtained.

mp 130-133 °C (i-PrOH), [α]₀^{26} +43.1 (c 2, CHCl₃); lit.⁵ 131-132 °C, [α]₀ +45.1 (c 1.32 CHCl₃). ¹H (CDCl₃): 8.01-7.32 (15H), 6.44 (1H, s), 5.91 (1H, dd, J 5.0 Hz and 6.4 Hz), 5.79 (1H, d, J 4.8 Hz), 4.82-4.75 (2H, unresolved), 4.52 (1H, dd, J 5.7 Hz and 13.9 Hz), 2.00 (3H, s). ¹³C (CDCl₃): 169.2, 166.1, 165.5, 165.2, 133.8, 133.7, 133.4, 130.0, 129.9, 128.7, 128.6, 98.6, 80.2, 75.2, 71.6, 63.9, 21.0.

5-Methyluridine 1

A. via 2,3,5-tri-O-benzoyl-5-methyluridine 17. Thymine (35.9 g, 297 mmol), HMDS (400 mL) and (NH₄)₂SO₄ (0.5 g) were refluxed under argon until a clear solution was obtained (ca. 4 h). Excess of the reagent was evaporated. Co-evaporation with toluene was performed following by drying on an oil pump. CH₃CN (700 mL) was added, followed by 15 (145 g, 288 mmol). The flask was immersed in ice-water bath and SnCl₄ (76 mL, 167 g, 649 mmol) was added using a syringe while maintaining magnetic stirring. After 1 h counting from the end of addition, the cooling bath was removed and the reaction proceeded at rt. After a total time of 6 h TLC showed that all 15 had reacted to form a more polar product 17 (Rf 0.85 and 0.31, respectively, in hexane-EtOAc, 2:1). The mixture was transferred to a separatory funnel charged with CH₂Cl₂ (700 mL) and ice-cold water (4 L), and extraction was performed. The organic layer was washed again with water, dried and the volatiles were evaporated. The hard crystalline mass thus obtained weighed 182 g. A small amount crude 17 was purified by chromatography in hexane-EtOAc, 2:1 to get an analytical sample. The abovementioned mass was dissolved in 1,4-dioxane (200 mL) with warming, and MeOH (600 mL) was added followed by few pieces of sodium and the mixture was left over the weekend. Amberlite IRA 120 H⁺ was added to remove the Na cations, and was filtered off and washed with MeOH. Most of the volatiles were evaporated to a point that the...
crystallization did not start. Water was added followed by CHCl₃ and extraction was performed three times to remove methyl benzoate. The water phase was evaporated until turbidity and was incubated in a refrigerator for 24 h. To the resulting semi-crystalline mass was added small volume of cold 96% EtOH. The crystals were removed by filtration, washed twice with cold 96% EtOH and with Et₂O. Final drying on oil pump gave 1 (67 g, ca. 90% for two steps). 17: mp 161-164 °C (EtOH); lit.⁴⁸ mp 163-164 °C. Exact mass: calc. for [C₃₁H₅₁N₂O₉ + Na⁺]⁺ = 593.1531, found: 593.1523. ¹H (CDCl₃): 9.59 (1H, exchangeable), 8.14-7.34 (15H, four groups of signals), 7.17 (1H, unresolved q, J 1Hz), 6.43 (1H, d, J 6 Hz), 5.92 (1H, dd, J 3 Hz and 6 Hz), 5.77 (1H, t, J 6 Hz), 4.87 (1H, dd, J 3 Hz and 12 Hz), 4.71-4.46 (1H, unresolved), 4.63 (1H, d, J 6 Hz), 1.58 (3H, unresolved d, J 1 Hz). ¹³C (CDCl₃): 165.9, 165.3, 163.7, 150.4, 134.9, 133.7, 133.6, 133.5, 129.8, 129.7, 129.5, 129.1, 128.7, 128.5, 128.4, 112.1, 86.9, 80.4, 73.3, 71.3, 63.9, 12.0.

B. Via 2,3,5-tri-O-acetyl-5-methyluridine 18. Thymine (8.63 g, 77 mmol), hexamethyldisilazane (100 mL) and (NH₄)₂SO₄ (0.1 g) were refluxed under argon until the solution became clear (ca. 3 h). Excess of the HMDS was removed by evaporation and the oily residue was dried on an oil pump. Vacuums were broken using balloons with argon. To the residue was added CH₃CN (400 mL), followed by 16 (98% pure, 25 g, 77 mmol) and SnCl₄ (19.9 mL, 169 mmol) added via a syringe. The mixture was stirred magnetically during 11 h. TLC showed that 16 was consumed and more polar 18 was formed (Rf 0.65 and 0.18, respectively, in hexane-EtOAc, 1:1). Most of CH₃CN was evaporated below 30 °C and the residue was transferred to a separatory funnel charged with CH₂Cl₂ and ice cold water, and extraction was performed. The organic layer was washed with water, dried and the volatiles were evaporated to give a sticky oil. A small amount of this material was purified by chromatography in hexane-EtOAc, 1:1 to get a sample for analysis. The bulk of the crude extract was dissolved in 1,4-dioxane (50 mL) and MeOH (300 mL). A piece of Na was added and the mixture was left overnight. Amberlite IRA 120 H⁺ was added and 15 min later was removed by filtration on sintered glass and washed with MeOH. Evaporation of the volatiles was conducted until spontaneous crystallization started. The flask was left in a refrigerator overnight. To a semi-solid mass was added cold 96% EtOH and the crystals were filtered off, washed with cold 96% EtOH and finally with Et₂O. Drying on oil pump furnished 1 (14.1 g, 69.5% for two steps). 18: syrup; [α]₀²⁶ -10.5 (c 1, CH₂Cl₂), lit.⁷⁵ [α]₀ -14.6 (c 0.40 EtOH). ¹H (400 MHz, CDCl₃): 9.45 (1H, bs, exchangeable), 7.17 (1H, bs), 6.05 (1H, apparent d), 5.32 (2H, apparent d, J 4 Hz), 4.34-4.30 (3H, unresolved), 2.13, 2.10, 2.07 three s, 3H each, 1.91 (3H, unresolved d, J 1 Hz). ¹³C (100 MHz, CDCl₃): 170.1, 169.7, 163.6, 150.5, 134.9, 111.9, 86.9, 79.8, 72.5, 70.3, 63.2, 20.7, 20.5, 20.4, 12.6.

1,2;5,6-Di-O-isopropilidene-α-L-glucofuranose 27 and 2,3;5,6-di-O-isopropilidene-α-L-mannofuranose 28 via 1-deoxy-1-nitro-L-glucitol 21 and 1-deoxy-1-nitro-L-mannitol 22. To a solution of NaOMe prepared from Na (3.7 g, 0.16 mol) and MeOH (200 mL) was added CH₃NO₂ (60 mL, 68.2 g, 1.1 mol) and L-arabinose (15 g, 0.1 mol). The whole was stirred magnetically under a protecting atmosphere of dry argon during 18 h. The precipitated sodium salts of the nitroglicotil and nitromannitol were filtered off, washed with cold MeOH, dissolved in cold (ca. 5°C) water 100 mL, and this solution was added dropwise during 10 min to a solution of conc. H₂SO₄ (20 mL) in water (25 mL) at rt. 5 min after the end of addition, neutralization was performed using a warm (ca. 45 °C) solution of Ba(OH)₂. Precipitated BaSO₄ was removed by filtration through a bed of Celite. The filtrate as demineralized through passage through a column of 200 mL of the Amberlite IRA 120 H⁺ followed by the Amberlite IRA 410 OH⁻. The resins were washed with water (2 x 100 mL). The combined
aqueous solutions were evaporated and the glassy residue obtained was dried on oil pump overnight. Acetone (250 mL) was added followed by conc. H₂SO₄ (7 mL) and the mixture was subjected to ultrasound in a domestic cleaning bath for 50 min. This was performed in round-bottom flask even though it was recommended to use flat-bottom flasks due to smaller dispersion of the ultrasound. TLC showed the L-manno compound 28 Rf 0.46 and more polar L-glucu compound 27 Rf 0.40 (in hexane-EtOAc, 2:1). The reaction mixture was neutralized with cold conc. NH₄OH and precipitated (NH₄)₂SO₄ was removed by filtration through Celite. The solids were washed with acetone and the combined brownish solutions were evaporated to leave material (2 g) which was separated using vacuum-dry chromatography (hexane-EtOAc, 2:1) to yield less polar 28 (0.75 g) and more polar 27 (0.43 g). Due to the presence of impurities, no effort was made to isolate 1,2-O-isopropylidene-L-gluconanose invariably present during acetonation of glucose.

**A.** To a solution of 27 (2.1 g) furnished 29 (1.45 g, 69%). Mp 74-77 °C (hexane-EtOAc), [α]₀° -34.5 (c 2 CHCl₃), lit. mp 78-79° C, [α]₀° -36.2 (c 0.5 CHCl₃). Exact mass: calc. for [C₁₂H₂₀O₆]⁺ = 260.1259, found: 260.1254; calc. for [C₁₂H₂₀O₆ + Na]⁺ = 283.1152, found: 283.1149. ³H (400 MHz, CDCl₃): 5.81 (1H, d, J 4.0 Hz), 4.92 (1H, dd, J 3.7 Hz and 5.9 Hz), 4.61 (1H, d, J 5.9 Hz), 4.33-4.38 (2H, m), 4.18 (1H, dd, J 3.6 Hz and 7.1 Hz), 4.08 (1H, dd, J 5.9 Hz and 8.7 Hz), 4.05 (1H, dd, J 5.0 Hz and 8.7 Hz), 3.27 (1H, exchangeable, J 2.5 Hz), 1.47, 1.46, 1.36, 1.32 four s, 3H each. ¹³C (100 MHz, CDCl₃): 112.6, 109.1, 101.2, 85.4, 80.1, 79.6, 73.2, 66.5, 26.8, 25.8, 25.1, 24.4.

**B.** By degradation of 29. Following the directions for the transformation of 9 into 12 using the Conditions B and proportional quantities of the reagents, L-allose 29 (1.1 g) was transformed into 32 (0.76 g, 69%) after chromatography in CH₂Cl₂ - MeOH, 20:1.

**C.** By desilylation of 37. To a solution of 37 (13.2 g, 38.8 mmol) in THF (50 mL) was added 1 M Bu₄NF in THF (41 mL). After 3 h, TLC showed that the substrate 37 (Rf 0.51, hexane-EtOAc 4:1) had been consumed to form a product 32 Rf 0.41 (CH₂Cl₂-MeOH 20:1). The solvent was evaporated and residual oil was purified by chromatography (CH₂Cl₂-MeOH 20:1) to give 32 (7.3 g, 89%). Mp 83-86 °C (hexane-EtOAc), [α]₀° -42.0 (c 2.2, CH₂Cl₂); lit. 82-84 °C, [α]₀° -45.3 (c 1.00 CH₂Cl₂). Exact mass: cal. for [C₆H₁₂O₅ + Na]⁺ = 213.0733, found: 213.0740. NMR (CDCl₃): 5.81 (1H, d, J 3.7 Hz), 4.58 (1H, t, J 4.4 Hz), 4.32-3.96 (2H, m), 3.84 (1H, dt, J 3.1 Hz and 3.1 Hz and 4.4 Hz), 3.74 (1H, dd, J 3.6 Hz and 12.2 Hz), 2.47 (bs), 1.57, 1.37 3H each, two s. ¹³C (CDCl₃): 112.7, 103.9, 80.6, 78.7, 70.8, 60.8, 26.5, 26.4.

**1,2-O-isopropylidene-α-L-xylofuranose 34.** To a magnetically stirred suspension of L-xylose (15 g, 100 mmol) in acetone (400 mL) was added dropwise conc. H₂SO₄ (15 mL, 27.6 g, 181 mmol) during 5 min. After 30 min counting from the end of addition, the mixture was cooled in ice-water bath and a solution of Na₂CO₃ (20 g, 189 mmol) in H₂O (190 mL) was slowly added while maintaining the internal temperature below 20 °C. After 2.5 h TLC showed nearly complete hydrolysis of the bis-acetonide 33 Rf ca 0.90 and formation of the diol 34 Rf
0.45 (CH₂Cl₂-MeOH 20:1). More Na₂CO₃ (10 g) was added to effect a complete neutralization. After stirring for 10 min the solid material was removed by filtration through sintered glass and was washed with acetone. Evaporation of acetone and chromatography in CH₂Cl₂-MeOH 20:1 furnished 34 (14 g, 74%); mp 37-38 °C; [α]₀⁺26 = +17 (c 3, CHCl₃); lit. for the D enantiomer: oil, [α]₀ -13.9 (c 0.34 CHCl₃). Alternative approach to get 34 was reported in the ref. 78. NMR¹H (DMSO-d₆): 5.79 (1H, d, J 3.7 Hz), 5.13 (exchangeable, d, J 4.7 Hz), 4.61 (exchangeable, t, J 5.6 Hz), 4.36 (exchangeable, d, J 3.7 Hz), 4.00-3.92 (2H, unresolved), 3.64-3.57 (1H, m of five lines, J 5.6 Hz), 3.54-3.46 (1H, m of five lines, J 5.6 Hz), [after D₂O exchange: 3.62 (dd, J 5.0 Hz and 11.2 Hz; 3.50 (dd, J 6.1 Hz and 11.2 Hz)], 1.38 and 1.23 (two s, 3H each). ¹³C (DMSO-d₆): 110.2, 104.2, 85.0, 81.3, 73.4, 58.8, 26.6, 26.1.

5-O-tButyldimethylsilyl-1,2-O-isopropylidene-α-L-xylofuranose 35. The title compound was obtained as an oil following the published procedure₇⁹ in higher yield, 90% vs. 75%. [α]₀⁺26 +9.1 (c 4, CHCl₃); lit. [α]₀ +11 (c 5.4 CHCl₃). ¹H (400 MHz, CDCl₃): 5.96 (1H, d, J - 0.8 Hz), 4.50 (1H, d, J 4.0 Hz), 4.37 and 4.33 (1H each, bs), 4.15-4.10 (3H, unresolved), 1.48 and 1.32 (3H each, two s), 0.90 (9H, s), 0.11 (6H, s). ¹³C (100 MHz, CDCl₃): 111.5, 105.0, 85.6, 78.1, 77.1, 62.3, 26.8, 26.1, 25.7, 18.1, -5.5, -5.7.

5-O-tButyldimethylsilyl-1,2-O-isopropylidene-α-L-ribofuranose 37. To a magnetically stirred suspension of CrO₃ (15.8 g, 158 mmol) in dry CH₂Cl₂ (250 mL) was added pyridine, (25.4 mL, 25 g, 316 mmol). The mixture warmed and 30 min later a solution of 35 (16 g, 52.6 mmol) in CH₂Cl₂ (70 mL) was added followed by Ac₂O (14.9 mL, 16.1 g, 158 mmol). Slight warming took place again. 10 min later the reaction was quenched by addition of toluene – EtOAc mixture (1:1, 300 mL). The supernatant was drained and the black residue was washed with the same solvent mixture. The combined solutions were passed through a bed of silica gel prepared in toluene – EtOAc, 1:2. The fractions containing the ulose 36 Rr 0.44 (hexane – EtOAc 17:3) were pooled together and the volatiles were evaporated. Co-evaporation with toluene was performed to expel residual pyridine. After brief drying on an oil pump, 96% EtOH (150 mL) was added and the solution was cooled in an ice-bath. NaBH₄ (5.1 g, 135 mmol) was added in one portion while maintaining magnetic stirring. 2 h later TLC showed the product 37 Rr 0.51 (hexane – EtOAc 4:1). Acetone (10 mL) was added to destroy the excess of NaBH₄ and 30 min later most of the volatiles were removed by evaporation. The residue was taken up in CH₂Cl₂ and washed with water. After conventional work-up (drying, flirtation, evaporation) and chromatography in hexane – EtOAc, 5:1, 37 as an oil (12.1g, 75.6% for two steps) was obtained.

Exact mass: cal. for [C₁₄H₂₈O₅Si + H]⁺ = 305.1779, found: 305.1778.

[α]₀⁺26 = -27.3 (c 2.1 CHCl₃); lit. for the D enantiomer: [α]₀ +30.7 (c 1.1 CHCl₃). NMR¹H (400 MHz, CDCl₃): 5.79 (1H, d, J 3.8 Hz), 4.56 (1H, dd, J 4.1 Hz and 4.9 Hz), 3.99 (1H, ddd, J 5.2 Hz and 7.9 Hz and 9.5 Hz), 3.91 (1H, dd, J 4.1 Hz and 12.6 Hz), 3.84-3.78 (2H, unresolved), 2.41 (1H, exchangeable, J 9.6 Hz), 1.57, 1.37 two s, 3H each, 0.89 (9H, s), 0.08 and 0.07 (two s, 6H). ¹³C (100 MHz, CDCl₃): 112.5, 104.2, 81.2, 78.8, 71.2, 61.8, 26.6, 25.9, 18.4, -5.3, -5.4.

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