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Anomeric stereospecific synthesis of 2′-*C*-methyl β-nucleosides; the Holy reaction of cyanamide with 2-*C*-methyl-D-arabinose

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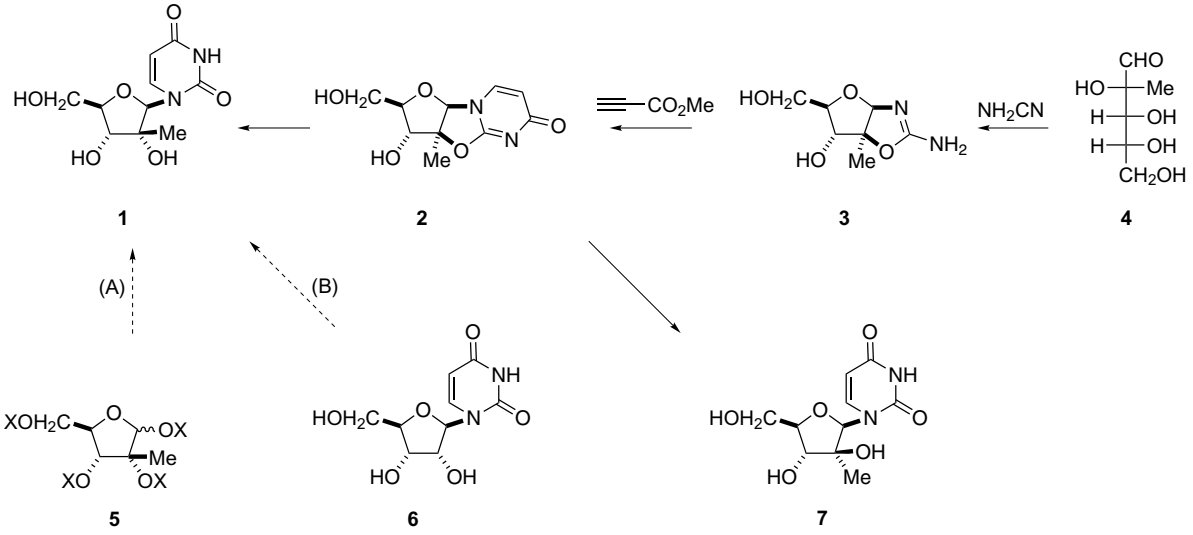
Abstract—The sequential reactions of 2-*C*-methyl-D-arabinose with cyanamide and methyl propiolate afford an anhydronucleoside, which may be opened under acid conditions with inversion at C2′, to give 2′-*C*-methyl uridine; ring opening with sodium hydroxide gave 2′-*C*-methyl *arabino*-uridine with retention of configuration at C2′. This gives complete stereospecific control to yield only β-nucleosides.

Although nucleosides with methyl branches on the sugar moiety have been known for a long time,[1] a number of 2′-*C*-methyl nucleoside analogues have been identified as promising therapeutics against hepatitis C virus NS5B RNA-dependent RNA polymerase.[2] In particular Valopicitabine (NM283), a valine ester of 2′-*C*-methyl cytidine, is in phase IIb clinical trials for the treatment of hepatitis C.[3,4]

Previous strategies for the synthesis of 2′-*C*-methyl nucleosides, illustrated by approaches to 2′-*C*-methyl uridine **1** (Scheme 1) have been (A) the coupling of a



Scheme 1.

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suitable protected activated derivative of a branched sugar **5** [with problems of anomeric specificity][3,5] and (B) the introduction of a methyl group to a β-nucleoside **6** [which requires a number of protections and functional group manipulations].[6,7] The Holy reaction of cyanamide with arabinose[8] and other sugars[9] allows the specific generation of pyrimidine β-nucleoside analogues;[10,11] the reaction of cyanamide with sugar precursors has been suggested as a prebiotic route to nucleic acids.[12] This Letter presents a new strategy for the synthesis of 2′-*C*-methyl uridine **1**; reaction of 2-*C*-methyl arabinose **4** with cyanamide afforded oxazoline **3**, which upon treatment with methyl propiolate gave the 2′-*C*-methyl anhydronucleoside **2**. The tricyclic system **2** can be opened with the hydroxide ion to give the *arabino*-branched nucleoside **7** whereas benzoylation followed by acid treatment predominantly gives the target 2′-*C*-methyl uridine **1**.

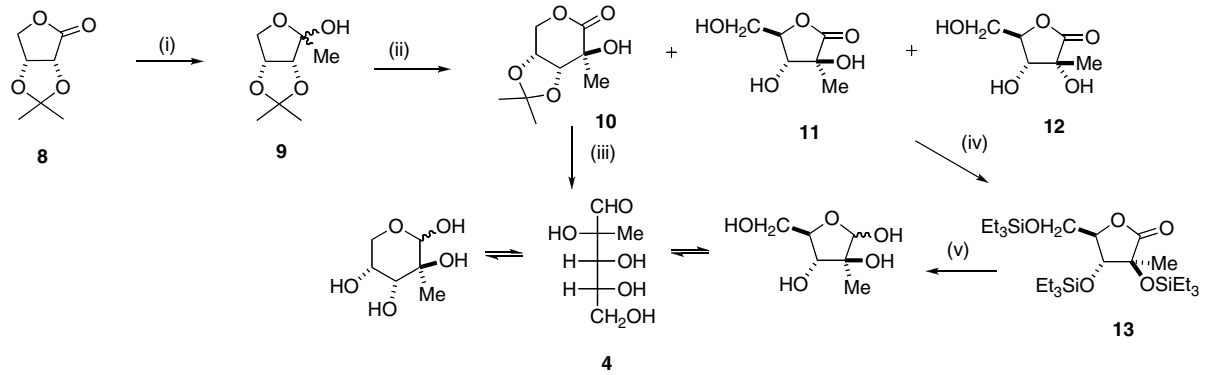
The synthesis of 2-*C*-methyl arabinose **4** is shown in Scheme 2. The Kiliani reaction of cyanide on ketohexoses[13,14] provides easy access to branched carbohydrate chirons. Reaction of methyl magnesium bromide with the protected D-erythronolactone **8** afforded the protected 1-deoxy ribulose **9** in 99% yield. The 1-deoxyketopentose **9** with sodium cyanide underwent a highly diastereoselective Kiliani ascension[15] to give the protected arabinono-1,5-lactone **10**[16] together with the unprotected arabinono-1,4-lactone **11**[17] and a small amount of the epimeric ribono-lactone **12**. The combined yield of **10** and **11** was 60%; as the acetonide in **10** can easily be hydrolysed to give **11**, the relative proportions of **10** and **11**, generated in the Kiliani reaction, depend on the precise reaction conditions. The protected lactone **10** was easily isolated by column chromatography, but the two lactones, **11** and **12**, were not easy to separate—but serendipitously **11** was found to crystallise out of the isomeric lactone mixture.

Both **10** and **11** can be used to access the desired lactol **4** (Scheme 2). Protection of **11** with triethylsilyl groups was required prior to reduction with DIBALH to give lactol **13** in 97% yield. This was subsequently deprotected using Dowex resin (50W X8 (H^+)) to give **4** in a quantitative yield. Alternatively, the 3,4-*O*-isopropylidene protected γ-lactone **10** was converted in two steps, reduction to the lactol using DIBALH followed by deprotection with Dowex resin (50W X8 (H^+)), to the same lactol **4** in 49% yield over two steps. The unprotected branched arabinose **4** is a complex mixture of furanose and pyranose forms; full details of the NMR of 2-*C*-methyl arabinose **4** will be discussed elsewhere. Although at present the availability of the branched sugar 2-*C*-methyl-D-arabinose is limited to chemical syntheses such as this, advances in biotechnology and the production of rare monosaccharides by Izumoring[18] and other green techniques suggest that a wide range of such rare and novel monosaccharides could be readily accessible.

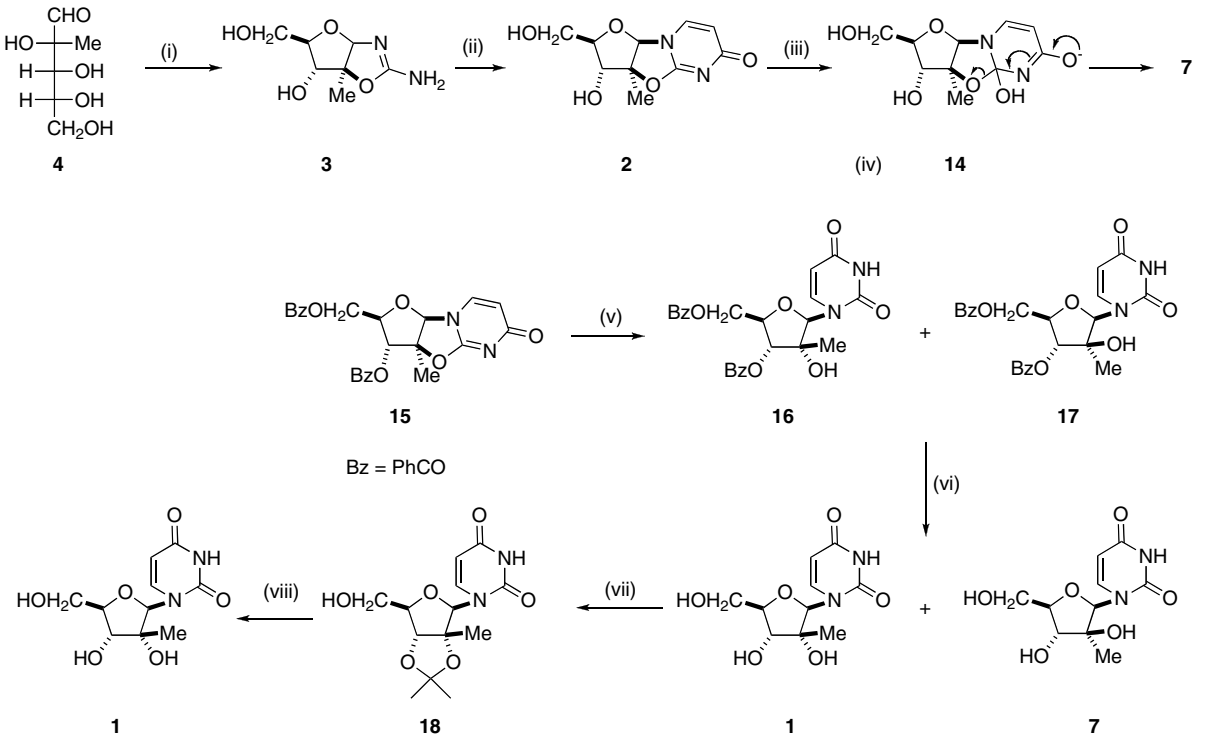
2-*C*-Methyl arabinose **4** with cyanamide and aqueous ammonia underwent a Holy reaction to form oxazoline **3**[19] in 62% yield; subsequent reaction of **3** with methyl propiolate in the presence of DBU gave the 2′-*C*-methyl-anhydrouridine **2**[20] (Scheme 3). There were various ambiguities about the structure of **2**, such as the sugar moiety could be in a pyranose or furanose form; the structure of the anhydronucleoside **2** was confirmed by X-ray crystallographic analysis.[21]

Treatment of anhydronucleoside **2** with sodium hydroxide in methanol gave the branched *arabino*-nucleoside **7**[22,7] in quantitative yield. Nucleophilic addition of hydroxide ion at C2 of the pyrimidine ring of **2** afforded adduct **14**, which fragmented to **7** without cleavage of the sugar C2′–O bond, thus retaining the configuration at C-2′.

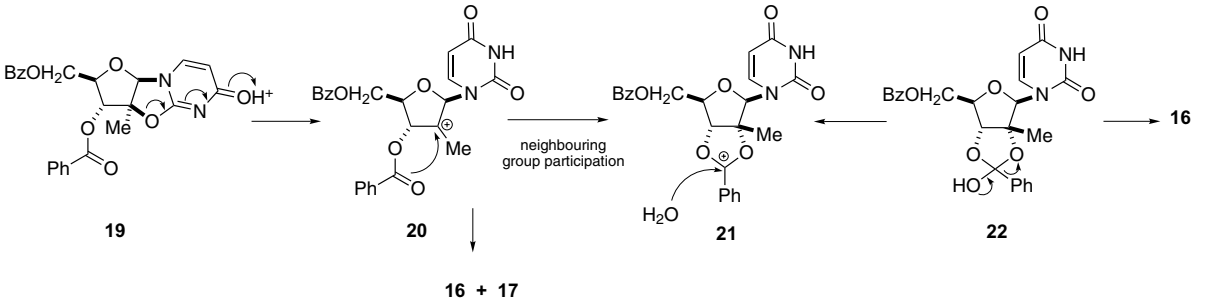
Conversion of the anhydronucleoside **2** to uridine **1** required inversion of configuration at C-2 of the sugar; S_N1 displacement would proceed via a tertiary carbocation at C-2 stabilised by the methyl branch. Numerous conditions were investigated and benzoyl protection of the anhydronucleoside allowed a satisfactory procedure. Esterification of **2** with benzoyl chloride in pyridine gave the dibenzoate **15** [mp 221–223 °C; $[\alpha]_D^{20}$ −55.5 (*c* 0.96 in CH_3CN)] in 98% yield. Treatment of **15** with *para*-toluenesulfonic acid in acetonitrile gave a quantitative yield of the inseparable dibenzoates **16** and **17**. Deprotection of the benzoyl protecting groups in **16** and **17** by sodium methoxide in methanol gave the *ribo*-**1** and the *arabino*-**7** branched nucleosides in a ratio of 11/1 (calculated from integration in the ^1H NMR spectrum). The epimers were not easy to separate by chromatography so the mixture was treated with acetone in the presence of anhydrous



Scheme 2. Reagents and conditions: (i) MeMgBr, THF, −78 °C; (ii) NaCN, H_2O; then H^+; (iii) $isoBu_2AlH$, CH_2Cl_2 −78 °C; then Dowex (H^+), H_2O; (iv) Et_3SiCl, imidazole, DMF; (v) $isoBu_2AlH$, CH_2Cl_2 −78 °C; then Dowex (H^+), dioxane–H_2O.



Scheme 3. Reagents and conditions: (i) NH_2CN, concd NH_4OH, 80 °C; (ii) methyl propiolate, 50% aq EtOH, DBU, reflux; (iii) NaOH, MeOH; (iv) BzCl, Pyr; (v) *p*-toluenesulfonic acid, MeCN, reflux; (vi) NaOMe, MeOH; (vii) $CuSO_4$, concd H_2SO_4 acetone; (viii) Dowex (H^+), H_2O.



Scheme 4.

copper sulfate and sulfuric acid to form the easily purified acetonide **18** [oil, $[\alpha]_D^{20}$ +32.2 (*c* 1.23 in CH_3CN)] in 74% over three steps. Removal of the acetonide in **18** by treatment with Dowex resin (H^+) in water gave the pure 2′-*C*-methyl uridine **1**[23,24] in 95% yield.

The excellent diastereoselectivity in the acid catalysed ring opening of the dibenzoyl anhydronucleoside **15** may be rationalised (Scheme 4). Initial protonation would give the cation **19** which might fragment to the tertiary carbocation **20** in which the C–O bond at C_2 had been broken. The tertiary cation **20**, stabilised by the methyl substituent could be attacked to give either of the epimers **16** and **17**. Alternatively the benzoyl group in **20** may act as a neighbouring group to form **21**, which would undergo capture by water to give **22** and would then ring open only to dibenzoate **16**.

In summary this Letter delineates a new strategy for the synthesis of 2′-*C*-methyl nucleosides via the ring opening of branched 2-*C*-methyl anhydronucleosides, ensuring that the stereochemistry of the anomeric position is completely defined. The likelihood of rare monosaccharides, such as 2-*C*-methyl arabinose **4**, being readily available in the near future from advances in biotechnology means that such antiviral nucleosides may be made by green chemistry.

Acknowledgements

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19. Oxazoline **3**: yellow oil, $[\alpha]_D^{20}$ −52.6 (*c* 1.255 in MeOH); ν_{max} (thin film): 3349 (s, br, OH), 1668 (s, NC=N), 1601 (m); δ_H (D_2O, 400 MHz): 1.38 (3H, s, Me), 3.55–3.59 (1H, dd, H5, *J* 6.2, 12.3), 3.68–3.72 (1H, dd, H5′, *J* 3.8, 12.3), 3.75–4.00 (1H, m, H4), 4.08 (1H, d, H3, *J* 6.3), 5.30 (1H, s, H1); δ_c (D_2O, 100 MHz): 16.6 (Me), 61.4 (C5), 76.5 (C3), 82.7 (C4), 95.2 (C2), 102.1 (Cl), 164.9 (NC=N).
20. Anhydro-2′-*C*-methyl undine **2**: mp 240–243 °C; $[\alpha]_D^{20}$ −52.0 (*c* 0.23 in MeOH); ν_{max} (thin film): 3332 (m, br, OH), 1651 (s, NC=O), 1522 (m, NC=O), 1479 (s); δ_H (MeOD, 400 MHz): 1.64 (3H, s, Me′), 3.51–3.56 (1H, dd, H5′, *J* 4.6, 12.0), 3.59–3.63 (1H, dd, H5″, *J* 3.7, 12.0), 4.06–4.09 (1H, a-dd, H4′, *J* 4.6, 8.4), 4.34 (1H, d, H3′, *J* 4.8), 5.90 (1H, s, H1′), 6.06 (1H, d, H5, *J* 7.4), 7.79 (1H, d, H6, *J* 7.4); δ_c (MeOD, 125 MHz): 17.1 (Me′), 61.9 (C5′), 77.4 (C3′), 89.4 (C4′), 95.3 (C1′), 97.6 (C2′), 109.6 (C5), 138.7 (C6), 161.4 (NC=N), 175.4 (C=O).
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22. 2′-*C*-Methyl *arabino*-uridine **7**: mp 132–135 °C [lit.[7] mp 137–138 °C]; $[\alpha]_D^{20}$ +79.7 (*c* 1.04 in MeOH); ν_{max} (thin film): 3418 (s, br, OH/NH), 1653 (s, HNC=O), 1448 (s); δ_H (D_2O, 400 MHz): 1.24 (3H, s, Me′), 3.71–3.76 (1H, m, H5′), 3.77–3.84 (1H, m, H5″), 3.85 (2H, m, H3′, H4′), 5.74 (1H, d, H5, *J* 8.2), 5.86 (1H, s, H1′), 7.75 (1H, d, H6, *J* 8.2); δ_c (D_2O, 100 MHz): 18.8 (Me′), 61.3 (C5′), 77.0 (C3′ or C4′), 79.4 (C2′), 83.7 (C4′ or C3′), 89.0 (C1′), 101.4 (C5), 143.6 (C6), 152.0 (C2), 166.5 (C4).
23. 2′-*C*-Methyl uridine **1**: mp 110–112 °C [lit.[24] mp 118–119 °C (softening at 101 °C)]; $[\alpha]_D^{20}$ +75.0 (*c* 1.25 in MeOH) [lit.[24] $[\alpha]_D^{20}$ +82 (*c* 0.7 in water)]; ν_{max} (thin film): 3390 (s, br, OH), 1694 (s, br, HNC=O); δ_H (MeOD, 400 MHz): 1.16 (3H, s, Me′), 3.77–3.81 (1H, dd, H5′, *J* 2.5, 12.5), 3.85 (1H, d, H3′, *J* 9.2), 3.91–3.95 (1H, ddd, H4′, *J* 2.2, 2.4, 9.2), 3.97–4.00 (1H, dd, H5″, *J* 2.1, 12.5), 5.70 (1H, d, H5, *J* 8.1), 5.96 (1H, s, H1′), 8.16 (1H, d, H6, *J* 8.2); δ_H (D_2O, 400 MHz): 1.16 (3H, s, Me′), 3.79–3.83 (1H, dd, H5′, *J* 4.2, 13.5), 3.86 (1H, d, H3′, *J* 9.2), 3.97–4.01 (2H, m, H4′, H5″), 5.84 (1H, d, H5, *J* 8.1), 5.95 (1H, s, H1′), 7.89 (1H, d, H6, *J* 8.2); δ_c (MeOD, 100 MHz): 20.2 (Me′), 60.4 (C5′), 73.3 (C3′), 80.1 (C2′), 83.8 (C4′), 93.1 (C1′), 102.3 (C5), 142.5 (C6), 152.5 (C2), 166.1 (C4); δ_c (D_2O, 100 MHz): 19.3 (Me′), 59.8 (C5′), 72.6 (C3′), 79.4 (C2′), 82.0 (C4′), 91.9 (C1′), 102.5 (C5), 141.8 (C6), 151.9 (C2), 166.4 (C4).
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