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Note

A simple access to the D-mannosidase inhibitor, 1-deoxymannojirimycin

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Dedicated to Professor Karl Dax on the happy occasion of his 60th birthday

Abstract

Crystalline 1,3,4,5-tetra-*O*-acetyl-6-bromo-6-deoxy-*keto*-D-fructose was prepared by reaction of 1,3,4,5-tetra-*O*-acetyl-D-fructopyranose with triphenylphosphane dibromide in dichloromethane. Subsequent deprotection followed by reaction of the free 6-bromodeoxyfructofuranose with sodium azide in *N*,*N*-dimethylformamide furnished the corresponding 6-azidodeoxyketose. Catalytic hydrogenation led to 1-deoxymannojirimycin in 27% overall yield from 1,3,4,5-tetra-*O*-acetyl-D-fructopyranose. This access is simple, inexpensive, high-yielding and clearly suitable for multigram preparations.

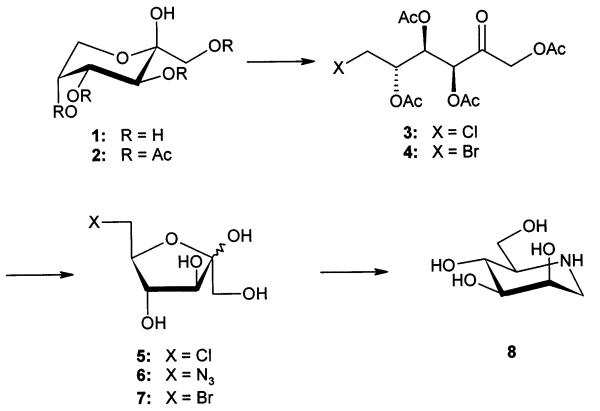
Keywords: 1-Deoxymannojirimycin; D-Mannosidase inhibitor; Open-chain ketose; 1,3,4,5-Tetra-*O*-acetyl-6-bromo-6-deoxy-*keto*-D-fructose

The natural product, 1-deoxymannojirimycin (1,5-dideoxy-1,5-imino-D-mannitol, **8**), is a potent inhibitor of various D-mannosidases.[1] In particular it is a specific inhibitor of Golgi mannosidase IA/B, rendering Golgi mannosidase II unaffected.[1] An O-glycosylated derivative was found to be a specific inhibitor of a Golgi-located endo-α-D-mannosidase.[2] In immobilised form, compound **8** has been employed in the affinity purification of mannosidases of glycoprotein trimming.[3]

Consequently, this product has become an important diagnostic tool in glycobiology with demand rapidly increasing at a fairly high price. Several synthetic approaches have been devised but, despite the community's strong interest, most of them are hampered by their lack of scaling-up potential.[4,5]

We have reported[4] a protecting-group-free, four-step approach to compound **8** from sucrose, which gave us convenient access to gram quantities but, due to difficulties with purification procedures at the 6,6′-dichlorodideoxysucrose stage, failed to provide a simple routine supply of desired amounts of 50 g and more.

In attempts to overcome the inconveniences of this synthesis, we identified 1,3,4,5-tetra-*O*-acetyl-6-chloro-6-deoxy-*keto*-D-fructose (**3**)[6] as a promising intermedi-



Scheme 1.

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ate (Scheme 1). This nicely crystalline material is easily available from D-fructose by sulfuric acid catalysed O-acetylation (55–60%)[7] and subsequent reaction of the resulting crystalline tetra-*O*-acetyl derivative (**2**) with PCl_5 in toluene (80%). Unfortunately, reaction on this protected open-chain ketose with sodium or tetrabutylammonium azide in a variety of solvents resulted in β-elimination reactions and subsequent extensive decomposition of the starting material, suggesting the introduction of the azido group at the stage of the free chlorodeoxyfructofuranose. Deprotection of compound **3** with sodium methoxide in methanol gave 6-chloro-6-deoxy-D-fructofuranose (**5**) in 80% yield. All attempts to convert free ketose **5** into 6-azido-6-deoxy-D-fructofuranose (**6**)[4] employing sodium or tetrabutylammonium azide in *N*,*N*-dimethylformamide, acetone, acetonitrile or mixtures of these solvents failed to produce more than a single-figure percentage of the desired azidodeoxyketose.

Consequently, we turned our attention to the recently prepared corresponding 6-bromo-6-deoxy sugar[8] to improve the leaving group at C-6. Reaction of compound **2** with PBr_3 in toluene led to the formation of the desired open-chain fructose **4**,[8] albeit in only 30% yield in our hands. Several attempts were made to improve the outcome of the bromodeoxygenation reaction, for example, by employing PBr_5. With this reagent, unstable 1,3,4,5-tetra-*O*-acetyl-D-fructopyranosyl bromides were formed exclusively. Gratifyingly, with commercially available triphenylphosphane dibromide in dichloromethane, practically quantitative formation of the desired open-chain bromosugar **4** took place, which by crystallisation from 2-propanol could be completely purified from triphenylphosphane oxide and isolated in 90% yield. Its deprotection gave syrupy 6-bromo-6-deoxy-D-fructofuranose (**7**), which turned out to be a suitable starting material for the desired intermediate **6**. The latter could be obtained from the reaction of free **7** with sodium azide in DMF at ambient temperature in 60–65% isolated yield, but due to the low reaction temperature required to avoid side reactions, reaction times of 6–8 days were found necessary.

Conventional catalytic hydrogenation of azidodeoxysugar **6** in dry methanol over Pd–C (5%) furnished compound **8**, which crystallised upon addition of ether to the concentrated methanolic solution of the free base and was isolated in yields around 70%. At this point it should also be noted that the syrupy azidodeoxysugar **6** should be purified on silica gel to allow for better yields and a cleaner reaction in the hydrogenation step.

Conveniently, this competitive sequence is based on abundant D-fructose and only a few, inexpensive reagents. It allows access to compound **8** in overall yields around 27% calculated from easily available 1,3,4,5-tetra-*O*-acetyl-D-fructopyranose (four steps) or of 15–20% for the five steps from D-fructose. Intermediates **2** and **4** are isolated by crystallisation. The approach is suitable for scaling up and, consequently, rivals any of the currently available procedures for the synthesis of 1-deoxymannojirimycin.

1. Experimental

General methods.—Melting points were recorded on a Tottoli apparatus and are uncorrected. Optical rotations were measured on a JASCO Digital Polarimeter or with a Perkin–Elmer model 341 polarimeter with a path length of 10 cm. NMR spectra were recorded at 200 as well as 300 MHz (^1H), and at 50.29 and 75.47 MHz (^{13}C). $CDCl_3$ was employed for protected compounds and D_2O for free sugars. Chemical shifts are listed in δ units employing residual protected, not deuterated, solvent as the internal standard. The signals of the protecting groups were found in the expected regions and are not listed explicitly. TLC was performed on precoated aluminum sheets (E. Merck 5554). TLC plates were stained with concd H_2SO_4 containing 5% vanillin.

TLC of iminoalditols was performed employing a mixture of 10% ammonium molybdate (w/v) in 10% aq H_2SO_4 containing 0.8% cerium sulfate (w/v).

For column chromatography Silica Gel 60 (E. Merck) was used. Mixtures of (1:10–3:1) EtOAc–petroleum ether were used for TLC of protected compounds. (3:1) Chloroform–MeOH was employed for TLC of unprotected sugars. Purification on silica gel was performed with 10:1 EtOAc–cyclohexane. The free inhibitor can be chromatographed in 100:100:1 $CHCl_3$–MeOH–concd aq ammonia.

Preparation of 1,3,4,5-tetra-O-*acetyl*-D-*fructopyranose* (**2**) *and 1,3,4,5-tetra*-O-*acetyl-6-chloro-6-deoxy*-keto-D-*fructose* (**3**).—1,3,4,5-Tetra-*O*-acetyl-D-fructopyranose (**2**) and 1,3,4,5-tetra-*O*-acetyl-6-chloro-6-deoxy-*keto*-D-fructose (**3**) were prepared by the reported methods.[6,7]

Preparation of 1,3,4,5-tetra-O-*acetyl-6-bromo-6-deoxy*-keto-D-*fructose* (**4**).—To a 5% solution of 1,3,4,5-tetra-*O*-acetyl-D-fructose (**2**, 52.0 g, 149.3 mmol) in dry CH_2Cl_2, pyridine (10 mL) and triphenylphosphane dibromide (82.5 g, 195.4 mmol, 1.3 equiv) were added, and the mixture was stirred under reflux for 3 h, then allowed to reach ambient temperature. Satd. aq sodium hydrogencarbonate was slowly added to destroy excess reagent. The organic layer was then consecutively washed with 5% aq HCl and 5% aq $NaHCO_3$. After drying over sodium sulfate, the solution was concentrated under reduced pressure. The remaining slightly yellow crystalline material was treated with hot 2-propanol, and the mixture was allowed to reach ambient temperature. The precipitate was collected and

again dissolved in hot 2-propanol and allowed to crystallise to remove residual triphenylphosphane oxide. Ketose **4** was thus obtained as colourless crystalline material (54.6 g, 89%): mp 112–113 °C; $[\alpha]_D^{20}$ +37.4° (*c* 1.2, $CHCl_3$). [Lit.[8]: mp 114 °C; $[\alpha]_D^{20}$ +44°, (*c* 1.0, $CHCl_3$)]. ^{1}H NMR ($CDCl_3$): δ 5.64 (1 H, dd, $J_{3,4}$ 2.0, $J_{4,5}$ 8.8 Hz, H-4), 5.46 (1 H, d, H-3), 5.24 (1 H, m, H-5), 4.90 (1 H, d, $J_{1a,1b}$ 17.4 Hz, H-1a), 4.68 (1 H, d, H-1b), 3.59 (1 H, dd, $J_{5,6a}$ 3.4, $J_{6a,6b}$ 11.4 Hz, H-6a), 3.43 (1 H, dd, $J_{5,6b}$ 5.7 Hz), H-6b); ^{13}C NMR: δ 198.0 (C-2), 74.2, 70.3, 69.0, 67.0 (C-1, C-3, C-4, C-5), 31.2 (C-6).

*Preparation of 6-bromo-6-deoxy-α,β-*D*-fructofuranose* (**7**).—To a 3% methanolic solution of bromodeoxyketose **4** (49.4 g, 120.1 mmol), 1 M methanolic sodium methoxide was added slowly at 0 °C until pH 8 was reached. The reaction was found to be completed after 5 h. Acidic ion-exchange resin (Amberlite IR 120 [H^+]) was washed with MeOH and added to the reaction mixture until neutral. After filtration, the solution was concentrated under reduced pressure, and the remaining yellow foam was purified on silica gel (1:20 cyclohexane–EtOAc) to give **7** (20.5 g, 70%) as a slightly yellow syrup: $[\alpha]_D^{20}$ +1.9° (*c* 1.8, MeOH); ^{13}C NMR (D_2O): δ 104.8, 101.9 (C-2, α/β), 82.1, 80.2, 79.6, 78.4, 77.0, 75.4 (C-3 α/β, C-4 α/β, C-5 α/β), 62.9, 62.8 (C-1 α/β), 33.9, 32.7 (C-6 α/β). Anal. Calcd for $C_6H_{11}BrO_5$: C, 29.65; H, 4.56. Found: C, 29.61; H, 4.59.

*Preparation of 6-azido-6-deoxy-α,β-*D*-fructofuranose* (**6**).—To a 3% solution of bromodeoxysugar **7** (19.1 g, 15.7 mmol) in *N*,*N*-dimethylformamide (DMF), sodium azide (94 g, 18.4 equiv) was added, and the mixture was stirred at ambient temperature for 7 days. Dichloromethane (1300 mL) was added, and the precipitate was removed by filtration. Concentration of the filtrate under reduced pressure furnished a yellow material that was purified on silica gel to give free azidodeoxyketose **6** (10.6 g, 66%) identical with previously prepared material;[4] $[\alpha]_D^{20}$ +42.1° (*c* 1.3, MeOH); ^{13}C NMR (D_2O): δ 102.5, 99.6 (C-2 α/β), 79.6, 77.6, 76.8, 74.8, 72.8, 72.7 (C-3 α/β, C-4 α/β, C-5 α/β), 60.6, 60.3 (C-1 α/β), 50.3, 49.3 (C-6 α/β).

*Preparation of 1,5-dideoxy-1,5-imino-*D*-mannitol (1-deoxymannojirimycin*, **8**).—A 2.5% methanolic solution of azidodeoxyfructose **6** (5.2 g, 25.3 mmol) was stirred with Pd–C (5%, 0.5 g) under an atmosphere of hydrogen at ambient temperature. The reaction was found completed after 4 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The remaining crude product was dissolved in water, and treated with basic ion-exchange resin (Merck III). After filtration and removal of the water under reduced pressure, MeOH was added to the yellow residue, and the solution was seeded to induce crystallisation. Slightly yellow product **8** was isolated by filtration (2.5–2.9 g; 60–70%). Additional product was obtained by conventional processing of the mother liquor. Physical data and spectra of **8** were identical with the data from previously prepared material.[4]

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