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Synthesis of 4-amino 3,4-dideoxy-D-*arabino*-heptulosonic acid 7-phosphate, the biosynthetic precursor of C_7N units in ansamycin antibiotics

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Abstract

The synthesis of 4-amino-3,4-dideoxy-D-*arabino*-heptulosonic acid 7-phosphate (**15**), the first committed intermediate in a new branch of the shikimate pathway of aromatic biosynthesis, is reported from 2-deoxy-D-glucose in 14 steps in 10–12% overall yield.

1. Introduction

Several families of antibiotics (e.g., ansamycins [1–3], mitomycins [4], ansamitocins [5]) contain a structural moiety, called a C_7N unit, consisting of a six-membered carbocycle carrying an extra carbon and a nitrogen substituent in a *meta* arrangement. Extensive tracer [2,3,6–8] and genetic [9–12] experiments have shown that this C_7N unit is biosynthetically derived from the shikimate pathway, although shikimic acid [2,6,13,14] itself or its precursor, 3-dehydroquinic acid [8], were not incorporated. Although it had been thought that this lack of incorporation might be due to impermeability of the cells to these precursors, this explanation was ruled out when in one case it was shown [14] that shikimic acid was incorporated into another part of the molecule but not into the C_7N unit. 3-Amino-5-hydroxybenzoic acid (AHBA) was identified as the proximate precursor of these C_7N units [3,5,15–17], and it was shown that the nitrogen is derived from the amide nitrogen of glutamine [18] and is attached to the carbon corresponding to C-5, not C-3 of shikimic acid [8,19–22]. Using this information we proposed the

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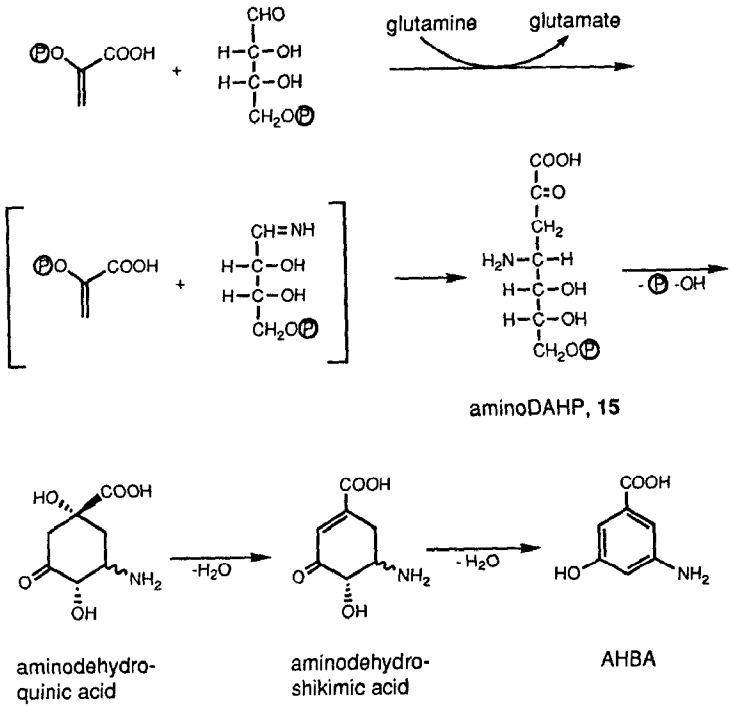


Scheme 1. Proposed mode of formation of 3-amino-3-hydroxybenzoic acid (AHBA), the precursor of mC_7N units, via 4-amino 3,4-dideoxy-D-*arabino*-heptulosonic acid 7-phosphate (aminoDAHP, **15**).

new variant of the shikimate pathway (Scheme 1) as the mode of formation of AHBA and hence the C_7N units [14,21].

The key molecule in this proposed pathway is 4-amino-3,4-dideoxy-D-*arabino*-heptulosonic acid 7-phosphate (aminoDAHP, **15**), already considered as a C_7N unit precursor by Hornemann et al. [8], the amino analogue of the first committed normal shikimate pathway intermediate, 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP), and an unknown compound. In order to test our hypothesis we required authentic **15**, and its chemical synthesis is the subject of the present report.

2. Results and discussion

The starting point of our synthesis of **15** (Scheme 2) was the preparation of DAHP reported by Frost and Knowles [23]. This route starts from 2-deoxy-D-glucose and produces **6** as an intermediate, a suitable starting point for our synthesis. However, since our synthetic route has substantially more total steps it was necessary to improve some of the component reactions and to adapt others to our strategy.

An alternate access to **6**, not used in this work, is the methylation of 3-deoxy-D-*arabino*-heptulosonic acid (DAH), the dephosphorylation product of DAHP, which accumulates in substantial amounts in fermentations of a genetically engineered strain of *E. coli* and can be isolated by ion-exchange chromatography as described by Frost and co-workers [24]. This is a viable alternative, given that even after extensive optimization the overall yield of the 5-step chemical synthesis of **6** from **1** was only ca. 35%.

In the chemical synthesis of **6**, the thioacetal **2** was obtained in 94% yield, using concentrated hydrochloric acid in ethanol, although a new mild method of thioacetalization has been published recently [25]. The fully protected sugar **3** was obtained in 95% yield by treatment with acidified acetone.

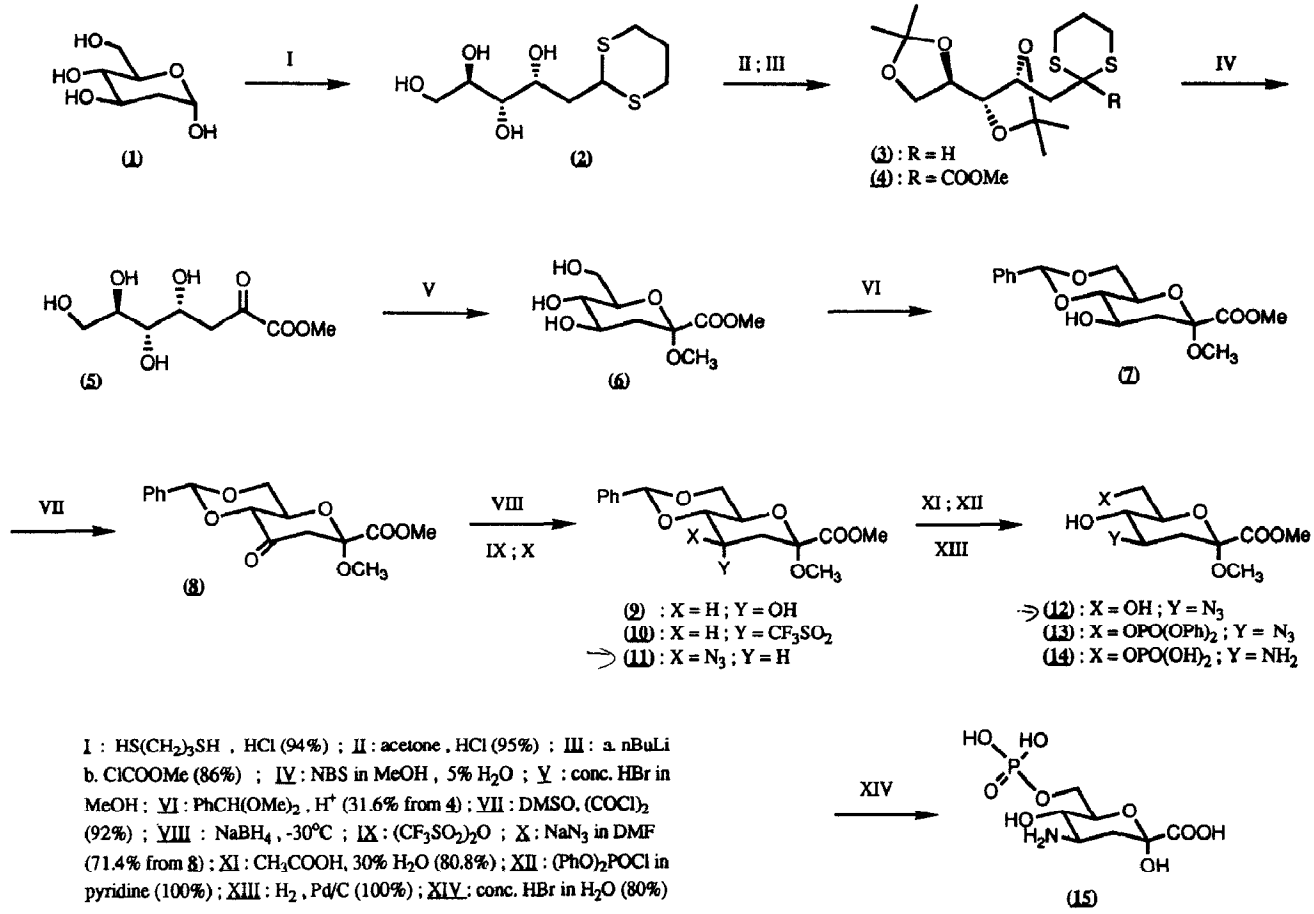
Although a new approach to the 'Umpolung reaction' was published during the course of this research [26], we developed our own method for the synthesis of the protected heptose **4**. In the presence of 12-crown-4-ether and a large excess of *n*-butyllithium, the intermediate carbanion was formed. It was transferred, at −78°C, to a solution of methyl chloroformate, avoiding any contact with the atmosphere. Product **4** was obtained in a satisfactory 86% average yield.

The removal of the 1,3-dithiane protecting group to give the corresponding ketone is very effectively accomplished using *N*-bromosuccinimide in the presence of water [27]. However, this reaction releases a large amount of hydrobromic acid which threatens the two ketal protecting groups present in compound **4**. To a lesser extent its methyl ester function could be affected too, especially as the deprotection reaction is usually carried out in solvents such as acetone or acetonitrile.

On the other hand, the cyclization of **5** to give **6** requires strongly acidic conditions. It was then logical to bypass intermediate **5** simply by adding some concentrated hydrobromic acid after the removal of the thioketal. This one-pot reaction (**4** → **6**) was done in aqueous (2.5%) methanol in order to protect the methyl ester function. It typically gave a 45% yield. The process was further streamlined by carrying out only a rough purification of **6** before reacting it with benzaldehyde dimethyl acetal [28] to give **7** in 31% overall yield from **4**.

Since the configuration at C-4 in **15** should be the same as in DAHP, it was necessary to invert the configuration of the secondary alcohol function **7** prior to its S_N2 displacement by nitrogen. This was done by an oxidation–reduction sequence. The Swern oxidation proceeded, according to the classical procedure [29], with a satisfactory 92% yield. The keto sugar **8** was found to be unstable even when stored at −20°C. It was reduced selectively to the axial alcohol at reduced temperature, which is required to obtain a selectivity of 9 to 1 or better in favor of the axial alcohol. The conversion was complete and the two stereoisomers may be separated at this stage or after the next step.

The conversion of an alcohol to an azide via a triflate [30] or a mesylate [31,32] with inversion of configuration has been widely used in carbohydrate chemistry. In order to introduce the azide function, the *ribo* derivative **9** was activated to the corresponding triflate **10**, which was not purified due to its instability. Displacement of the triflate by sodium azide [30] gave specifically, after purification, the



Scheme 2. Synthesis of 4-amino 3,4-dideoxy-D-*arabino*-heptulosonic acid 7-phosphate (**15**).

equatorial azide **11** in 71% yield (from **8**). The azide **11** was subsequently deprotected in aqueous acetic acid [33] to give the diol **12** in 81% yield.

The primary alcohol function of **12** was then selectively phosphorylated [33] with diphenyl chlorophosphate in quantitative yield to produce the azido diphenyl phosphate **13**. The secondary alcohol function does not react with the phosphate function to displace one of its phenoxy groups, as observed during the monophosphorylation of 1,2-syn diols [35] or, under basic conditions, with D-xylose 5-diphenylphosphate [34]. Product **13** was hydrogenated [34,36–38] in quantitative yield, but the reaction must be carefully monitored since overhydrogenation produces an unidentified impurity which is extremely difficult to remove. The last step, the hydrolysis leading to the final product (**15**), was somewhat problematic, but was finally achieved cleanly with 6.5 N hydrobromic acid. Attempts with concd HCl or with more dil HBr resulted in the sole cleavage of the methyl ester function or incomplete cleavage of the anomeric methoxy group. Indeed, both intermediates in the hydrolysis of the anomeric methoxy function are quite unfavored [39], due to the two strongly electron withdrawing groups, the phosphate and the ester group. Cleavage of the anomeric methoxy function with boron trichloride [40–42] was also explored as an alternative, but was dropped in favor of the simpler cleavage with HBr. This step completed the synthesis of the target compound, aminoDAHP **15**, in 14 steps with an overall yield of 10–12%.

As briefly reported elsewere [43], synthetic **15** was biologically active, serving as substrate for the enzymic synthesis of AHBA in a cell-free extract of the rifamycin producer, *Nocardia mediterranei*. It was also converted by *E. coli* dehydroquinate synthase into 5-deoxy-5-amino-dehydroquinic acid [44], another of the postulated intermediates of AHBA biosynthesis. The synthesis of **15** has thus made it possible to gather considerable biochemical support for the postulated mode of formation of the C_7N unit precursor AHBA shown in Scheme 1.

3. Experimental

2-Deoxy-D-arabino-*hexose propylene dithioacetal* (**2**).—2-Deoxy-D-glucose (20 g, 121 mmol) was added to 200 mL abs EtOH. The mixture was cooled to −35 to −40°C, and 200 mL concd HCl was added slowly without increase in the temperature. The 2-deoxy-D-glucose dissolved upon addition of the acid. 1,3-Propanedithiol (14.5 mL, 144 mmol) was added and the mixture was allowed to warm to room temperature. After 17 h the mixture was a thick paste which was filtered. The white solid was washed with abs EtOH, finely divided, and dried in a desiccator under vacuum to yield 20.1 g of **2**. The mother liquor was concentrated to ca. 15 mL and the grey precipitate was filtered and washed with EtOH until it was almost white. The solid was dried in a desiccator to give a further 7.4 g of **2**. A third crop of 1.7 g crystallized from the mother liquor at −20°C. The total yield of **2** was 29.2 g (114 mmol, 94.2%). This material was used without further purification.

2-Deoxy-3,4:5,6-di-O-*isopropylidene*-D-arabino-*hexose propylene dithioacetal* (**3**). —Compound **2** (29.16 g, 114 mmol) was covered with 600 mL of acetone. The mixture was cooled to −20°C and 24 drops of concd H_2SO_4 was added with vigorous stirring. The mixture was then warmed to room temperature and it became clear. After 36 h of reaction the solution was neutralized with concd NH_4OH (25 drops). The precipitate was removed by filtration over Celite and the filtrate was evaporated to leave a white solid. TLC (1:1 *n*-hexane–EtOAc, R_f 0.55) revealed the presence of a small amount of starting material. The crude product was purified by flash chromatography (1:1 *n*-hexane–EtOAc) in 5 batches to give 36.1 g of **3** as white crystals (108 mmol, 89% yield from 2-deoxy-D-glucose).

Methyl 3-deoxy-4,5:6,7-di-O-*isopropylidene*-D-arabino-*heptulosonate propylene dithioacetal* (**4**).—THF was dried by refluxing over sodium and distilled immediately prior to use. The glassware was dried and assembled in an oven and flushed with Ar while cooling. To a round-bottomed flask containing 12-crown-4 ether (2.6 g, 15 mmol) and THF (10 mL) was slowly added 2.5 M *n*-butyllithium (45 mL, 113 mmol) in THF to give a yellowish suspension. The mixture was cooled in an ice bath. The *n*-butyllithium suspension was then transferred into an addition funnel through glass tubing with Ar under pressure.

A solution of **3** (5 g, 15 mmol) in THF (150 mL) was prepared under Ar and cooled to −60°C. The *n*-butyllithium suspension was slowly added to **3** while maintaining the temperature below −55°C. When the addition was finished, the temperature was allowed to rise to −30°C. The color was deep purple at low temperatures but orange at −30°C. The solution was stirred for a total of 6.5 h including 3 h at −30 to −35°C. The solution was then cooled to −78°C and was added, with vigorous stirring, to a cooled (−78°C) solution of methyl chloroformate (46 mL, 595 mmol) in THF (45 mL). This was done through a glass tube fitted with a tap, using Ar under pressure. The rate of addition was regulated so that the temperature did not rise above −70°C. The glass bridge may be cooled with dry ice before the addition. The temperature was then gradually raised to −40°C and the mixture kept at that temperature for 45 min before allowing it to warm to room temperature. The mixture was concentrated under vacuum to a thick syrup; then CH_2Cl_2 was added and the lithium salts were removed by filtration. The filtrate was concentrated and subjected to flash chromatography (4:1 *n*-hexane–EtOAc). Compound **4** (R_f 0.33) was obtained in an average yield of 86% as a white to slightly yellowish amorphous solid. This procedure could be scaled up to produce at least 24 g of **3**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.18 (ddd, 1 H), 3.95–4.08 (m, 2 H), 3.88 (dd, 1 H), 3.72 (s, 3 H), 3.52 (dd, 1 H), 3.25 (ddd, 1 H), 3.03 (ddd, 1 H), 2.66 (m, 2 H), 2.45 (dd, 1 H), 2.35 (dd, 1 H), 2.05 (m, 1 H), 1.86 (m, 1 H), 1.36 (s, 3 H), and 1.29 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.0, 109.6, 109.5, 81.1, 76.6, 75.9, 67.3, 52.8, 52.7, 42.7, 27.9, 27.5, 26.9, 26.8, 26.64, 25.2, and 24.5.

Methyl (methyl 5,7-O-benzylidene-3-deoxy-D-arabino-*heptulopyranosid)onate* (**7**). —*N*-Bromosuccinimide (NBS) (103 g, 578 mmol) was partially dissolved in EtOH (1650 mL) containing water (40 mL) and the mixture was cooled to −20°C. Compound **4** (25.06 g, 63.92 mmol) in MeOH (400 mL) containing water (10 mL)

was added slowly to the NBS suspension. After several minutes the solution became red and after 3 h the suspension had become a clear solution. The mixture was left overnight at room temperature and was then pale orange.

The solution was diluted with MeOH (500 mL) and HBr [60 mL, 48% (w/w) in water] was added. This solution was refluxed for 1 day, when TLC (6:1 $CHCl_3$–MeOH) revealed the presence of **6** (R_f 0.50), along with other products, particularly one migrating just ahead of it. While the solution was cooling down, lead carbonate (180 g) was added with strong stirring. The stirring was continued until the temperature had decreased to 30°C. Then the mixture was filtered and the filtrate evaporated under vacuum. High boiling volatile products were removed by pumping under high vacuum at a bath temperature of 35°C. The solid residue was dissolved in MeOH and silica gel (25 g) was added. The MeOH was removed under vacuum and more high boiling volatiles were removed by high vacuum pumping, until the mixture had an almost dry appearance.

The residue was then worked into a slurry with 4:1 *n*-hexane–EtOAc, which was then poured onto a column of silica gel (45 × 7.5 cm). The column was eluted successively with 4:1 *n*-hexane–EtOAc, EtOAc (2.5 L), 7:1 $CHCl_3$–MeOH (4 L), and 6:1 $CHCl_3$–MeOH (1.5 L). Product **6** (11.2 g of an orange oil which crystallized after ca. 1 h) was eluted by the third solvent system almost free of succinimide. After elution with the last solvent, MeOH (60 mL) was added to the top of the column and the elution was resumed with 5:1 $CHCl_3$–MeOH until a dark band which had started to form upon the addition of MeOH had been collected. This second fraction was concentrated and chromatographed again ($CHCl_3$–MeOH) to give another crop of impure **6** (1.4 g) for a total of 12.6 g which was used in the next reaction. Pure **6** isolated from smaller scale experiments had the same characteristics as described [23].

Crude **6** (12.6 g, assumed to be 57.5 mmol) was dissolved in dry DMF (100 mL). The solution was stirred under aspirator vacuum at room temperature until bubbling ceased and the system was then flushed with Ar. Freshly distilled benzaldehyde dimethylacetal (10 mL, 66.4 mmol) was added, as well as a catalytic amount of *p*-toluenesulfonic acid, azeotropically dried with toluene. The mixture was again degassed by stirring at room temperature under aspirator vacuum. When bubbling had stopped, the solution was heated to 72°C under 35 mmHg pressure. After 4 h, only a very small amount of remaining starting material was observed by TLC (1.5:1 *n*-hexane–EtOAc). The reaction was then quenched with 20 drops of Et_3N and cooled to room temperature.

The solution was poured into $CHCl_3$ (650 mL) and washed with water (100 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated under vacuum. The crude brown oil was subjected to flash chromatography (1.5:1 *n*-hexane–EtOAc) to give a fraction containing product **7** (6 g, R_f 0.41), a fraction containing impure **7** (3.5 g), and a less polar fraction (3.5 g, R_f 0.63) resulting in part from overreaction of **6**.

This less polar fraction was hydrogenated [45] overnight in MeOH with palladium catalyst (10% Pd–C). The catalyst was filtered off through Celite and the filtrate was concentrated to leave a semisolid material, which was chro-

matographed on silica gel (1.5:1 *n*-hexane–EtOAc, then 4:1 $CHCl_3$–MeOH) to give 1.6 g of the triol **6**. This material was reacted again with benzaldehyde dimethylacetal following the previous procedure. The crude product was mixed with the impure fraction of **7** collected from the first chromatographic separation. It was subjected to further chromatography (identical conditions) and gave an additional 1.7 g of **7**. The combined product, 7.7 g of a white, sticky gum, after one more chromatographic purification (1.5:1 *n*-hexane–EtOAc) gave 6.6 g of **7** (20.2 mmol; 32% from **3**). HRFABMS: *m/z* 325.1272 $(M + H)^+$; calcd for $(C_{16}H_{20}O_7 + H)$ 325.1287. ^1H NMR (300 MHz, $CDCl_3$): δ 7.45 (m, 2 H, Ph, H-2′,6′), 7.34 (m, 3 H, Ph, H-3′,4′,5′), 5.55 (s, 1 H, PhC*H*), 4.32 (dd, 1 H, $J_{H\text{-}7eq,H\text{-}7ax}$ 10.2, $J_{H\text{-}7eq,H\text{-}6}$ 4.9 Hz, H-7*eq*), 4.18 (m, 1 H, H-4), 3.86 (t, 1 H, $J_{H\text{-}7ax,H\text{-}6}$ 10.3 Hz, H-7*ax*), 3.79 (s, 3 H, $COOCH_3$), 3.71 (dt, 1 H, H-6), 3.52 (t, 1 H, $J_{H\text{-}5,H\text{-}4} = J_{H\text{-}5,H\text{-}6} = 9.2$ Hz, H-5), 3.24 (s, 3 H, OCH_3), 2.60 (br s, 1 H, OH), 2.45 (dd, 1 H, $J_{H3eq,H\text{-}4}$ 5.2 Hz, H-3*eq*), 1.77 (dd, 1 H, $J_{H\text{-}3ax,H\text{-}3eq}$ 13.2, $J_{H3ax,H\text{-}4}$ 11.1 Hz, H-3*ax*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.9, 136.9, 128.9, 128.2, 127.7, 101.6, 99.1, 82.4, 68.3, 65.4, 64.0, 52.5, 50.5, and 39.3.

*Methyl (methyl 5,7-*O*-benzylidene-3-deoxy-4-oxo-*D*-*arabino*-heptulopyranosid)-onate* (**8**).—A three-neck flask, a thermometer, and two addition funnels were dried and assembled inside an oven and the apparatus was flushed with Ar. Dichloromethane was dried by refluxing over CaH_2 and was distilled just before use. Freshly distilled oxalyl chloride (5.41 mL, 62.14 mmol) was added to CH_2Cl_2 (54 mL) in the three-neck flask under Ar. The solution was cooled to −70°C. After 10 min Me_2SO (5.4 mL, 90.4 mmol) in CH_2Cl_2 (26 mL) was slowly added to the vigorously stirred solution. Five min after the addition, **7** (6.55 g, 20.2 mmol)in CH_2Cl_2 (165 mL) was slowly added and allowed to react for 45 min. Then Et_3N (18.9 mL) in CH_2Cl_2 (107 mL) was slowly added and the final solution was stirred for 15 min at −70°C, after which the mixture was allowed to warm to room temperature. The reaction was quenched with a mixture of $CHCl_3$ and water. The organic layer was dried over $MgSO_4$ and concentrated. The oily residue was subjected to column chromatography (10:1 CH_2Cl_2–EtOAc; **8**, R_f 0.69; **7**, R_f 0.29) to give **8** as a syrup (6.0 g, 18.7 mmol, 92% yield). This product had to be used within one or two days since it decomposed appreciably, even when stored at −20°C. ^1H NMR (300 MHz, $CDCl_3$): δ 7.47 (m, 2 H, Ph, H-2′,6′), 7.33 (m, 3 H, Ph, H-3′,4′,5′), 5.56 (s, 1-H, PhC*H*), 4.43 (dt, 1 H, $J_{H\text{-}6,H\text{-}5}$ $J_{H\text{-}6,7\text{-}ax}$ 5.65, $J_{H\text{-}6,H\text{-}7eq}$ 2.6 Hz, H-6), 4.36 (br dd, 1 H, $J_{H\text{-}7ax,H\text{-}7eq}$ 8.9, $J_{H\text{-}7ax,H\text{-}6}$ 4.5 Hz, H-7*ax*), 4.03 (d and dd, 2 H, $J_{H\text{-}5,H\text{-}6}$ 4.2, $J_{H\text{-}7eq,H\text{-}6}$ 3.2, $J_{H\text{-}7eq,H\text{-}7ax}$ 8.9 Hz, H-5,7*eq*), 3.82 (s, 3 H, $COOCH_3$), 3.28 (s, 3 H, CH_3O), 2.90 (d, 1 H, $J_{H\text{-}3eq,H\text{-}3ax}$ 14.6 Hz, H-3*eq*), 2.81 (dd, 1 H, $J_{H\text{-}3ax,H\text{-}7ax}$ 0.9 Hz, H-3*ax*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.6, 166.9, 136.2, 129.3, 128.2, 126.3, 102.0, 101.1, 82.2, 69.1, 66.3, 53.0, 51.3, and 48.1.

*Methyl (methyl 5,7-O-benzylidene-3-deoxy-*D*-ribo-heptulopyranosid)onate* (**9**).—Compound **8** (6.0 g, 18.7 mmol) was dissolved in dry MeOH (610 mL) and the solution was cooled to −25 to −30°C. Sodium borohydride (0.39 g, 10.3 mmol) was added in one portion and the solution was stirred at this temperature for 1.5 h until TLC (10:1 CH_2Cl_2–EtOAc) revealed no more starting material. A small amount of the equatorial alcohol **7** could be observed on TLC (10:1 CH_2Cl_2–

EtOAc; **9**, 0.32; **7**, R_f0.25: 1.5:1 *n*-hexane–EtOAc; **9**, R_f 0.22; **7**, R_f 0.28). The solution was diluted with $CHCl_3$ and washed twice with water. The combined aqueous phases were extracted three times with $CHCl_3$. The combined organic phases were dried over $MgSO_4$ and concentrated under vacuum. The remaining gum was subjected to chromatography (10:1 CH_2Cl_2–EtOAc) to give **9** (6.0 g, 18.52 mmol), containing up to 10% of **7** (estimated by NMR). The conversion was 99%, the yield of **9** was at least 90%. Compound **9** was purified on a small scale, but on a larger scale the two isomers were separated at a later stage. HRFABMS: *m/z* 325.1284 $(M+H)^+$; calcd for $(C_{16}H_{20}O_7+H)$ 325.1287. ^1H NMR (300 MHz, $CDCl_3$): δ 7.47 (m, 2 H, Ph, H-2′,6′), 7.33 (m, 3 H, Ph, H-3′,4′,5′), 5.59 (s, 1 H, PhC*H*), 4.37 (dd, 1 H, $J_{H\text{-}7eq,H\text{-}7ax}$ 10.3, $J_{H\text{-}7eq,H\text{-}6}$ 5.0 Hz, H-7*eq*), 4.18 (m, 2 H, H-6,4), 3.88 (t, 1 H, $J_{H\text{-}7ax,H\text{-}6}$ 10.3 Hz, H-7*ax*), 3.77 (s, 3 H, $COOCH_3$), 3.63 (dd, 1 H, $J_{H\text{-}5,H\text{-}4}$ 2.8, $J_{H\text{-}5,H\text{-}6}$ 9.7 Hz, H-5), 3.29 (s, 3 H, CH_3O), 2.92 (br s, 1 H, OH), 2.39 (dd, 1 H, $J_{H\text{-}3eq,H\text{-}4}$ 2.9, $J_{H\text{-}3eq,H\text{-}3ax}$ 14.9 Hz, H-3*eq*), 2.00 (dd, 1 H, $J_{H\text{-}3ax,H\text{-}4}$ 3.5 Hz, H-3*ax*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.9, 137.0, 129.0, 128.1, 126.1, 101.9, 98.9, 78.4, 68.9, 64.8, 59.8, 52.7, 51.3, and 37.6.

*Methyl (methyl 5,7-O-benzylidene-3-deoxy-4-trifluoromethanesulfonyl-*D-ribo*-heptulo-pyranosid)onate* (**10**).— A three-neck flask, a thermometer, and two addition funnels were dried and assembled inside an oven and flushed with Ar. The CH_2Cl_2 used was dried and distilled immediately before use. Trifluoromethanesulfonic anhydride (6 mL, 67.8 mmol) was dissolved in CH_2Cl_2 (180 mL) and the solution was cooled to −20°C. Pyridine (9.1 mL), stored over molecular sieves, was dissolved in CH_2Cl_2 (80 mL) and added slowly to the anhydride solution, which rapidly turned into a thick white paste. The flask was then manually agitated at −20°C and the addition was slowly continued. When the addition was complete, the contents of the flask had become a clear brownish solution. The temperature was maintained at −20°C and a solution of **9** (6.0 g, 18.5 mmol, containing up to 10% of **7**) in CH_2Cl_2 (170 mL) was slowly added with vigorous stirring. The mixture was stirred for 1.5 h at −20°C, then rapidly diluted with CH_2Cl_2 and washed twice with ice–water. The organic phase was dried with $MgSO_4$ and concentrated under vacuum at 20 to 25°C. The remaining pyridine was removed at room temperature under high vacuum to leave a red oil which was immediately used in the next reaction. TLC (2:1 *n*-hexane–EtOAc) **9**, R_f 0.12; **10**, R_f 0.28.

*Methyl (methyl 4-azido-5,7-O-benzylidene-3,4-dideoxy-*D-arabino*-heptulopyranosid)onate* (**11**).—The red oil from the previous reaction was dissolved at room temperature in dry DMF (350 mL, commercial grade stored over molecular sieves). Sodium azide (15 g, 231 mmol) was added in one portion. The solution was stirred for 2 h at room temperature, diluted with $CHCl_3$, and washed twice with water. The combined aqueous phases were extracted twice with $CHCl_3$ and the combined organic phases were dried over $MgSO_4$ and concentrated under vacuum. The oily residue was chromatographed twice (2:1 *n*-hexane–EtOAc; **11**, R_f 0.44) to give specifically the isomer **11** (4.7 g, 13.4 mmol) as a semisolid oil (71% yield from **8**). HRFABMS: *m/z* 350.1343 $(M+H)^+$; calcd for $(C_{16}H_{19}O_6N_3+H)$ 350.1352. ^1H NMR (300 MHz, $CDCl_3$): δ 7.47 (m, 2 H, Ph, H-2′,6′), 7.35 (m, 3 H, Ph, H-3′,4′,5′), 5.61 (s, 1 H, PhC*H*), 4.34 (dd, 1 H, $J_{H\text{-}7eq,H\text{-}7ax}$ 10.3, $J_{H\text{-}7eq,H\text{-}6}$ 4.5 Hz, H-7*eq*), 4.06

(ddd, 1 H, $J_{H\text{-}4,H\text{-}5}$ 9.4, $J_{H\text{-}4,H\text{-}3ax}$ 5.0, $J_{H\text{-}4,H\text{-}3eq}$ 11.6 Hz, H-4), 3.89 (t, 1 H, $J_{H\text{-}7ax,H\text{-}6}$ 10.2 Hz, H-7*ax*), 3.80 (s, 3 H, $COOCH_3$), 3.77 (m, 1 H, H-6), 3.62 (t, 1 H, H-5), 3.26 (s, 3 H, OCH_3), 2.41 (dd, 1 H, $J_{H\text{-}3eq,H\text{-}3ax}$ 13.5 Hz, H-3*eq*); 1.73 (dd, 1 H, H-3*ax*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.7, 136.8, 129.0, 128.2, 126.9, 101.5, 98.7, 81.1, 68.7, 64.9, 56.4, 52.8, 51.0, and 37.6.

*Methyl (methyl 4-azido-3,4-dideoxy-*D-arabino-*heptulopyranosid)onate* (**12**).—Compound **11** (4.65 g, 13.3 mmol) was dissolved in aq 70% AcOH (145 mL). The solution was heated to 39°C for 1 day. It was then concentrated under high vacuum while the temperature of the water bath was kept at 30°C. Remaining traces of AcOH were removed by azeotropic distillation with toluene (30 mL). The residue was purified by successive chromatography, first with 10:1 CH_2Cl_2–MeOH (R_f 0.42) and then with 5:2 CH_2Cl_2–acetone (R_f 0.35). White crystals (2.8 g, 10.8 mmol) of **12** were obtained in 81% yield; mp 100–102°C. HRFABMS: *m/z* 262.1031 $(M + H)^+$; calcd for $(C_9H_{15}O_6N_3 + H)$ 262.1039. ^1H NMR (300 MHz, methanol-d_4): δ 3.87 (d, 2 H, $J_{H\text{-}7,H\text{-}6}$ 3.0 Hz, H-7), 3.82 and 3.79 (ddd and s, 4 H, $J_{H\text{-}4,H\text{-}5}$ 9.0, $J_{H\text{-}4,H\text{-}3ax}$ 12.0, $J_{H\text{-}4,H\text{-}3eq}$ 4.9 Hz, H_4 and $COOCH_3$), 3.74 (br s, 2 H, 2 OH), 3.58 (dd, 1 H, $J_{H\text{-}5,H\text{-}6}$ 9.6, $J_{H\text{-}5,H\text{-}4}$ 9.0 Hz, H-5), 3.51 (dt, 1 H, H-6), 3.21 (s, 3 H, CH_3O), 2.34 (dd, 1 H, $J_{H\text{-}3eq,H\text{-}3ax}$ 13.2, $J_{H\text{-}3eq,H\text{-}4}$ 4.9 Hz, H-3*eq*), 1.64 (dd, 1 H, $J_{H\text{-}3ax,H\text{-}4}$ 12.1 Hz, H-3*ax*). ^{13}C NMR (75 MHz, methanol-d_4: δ 168.9, 98.0, 73.9, 69.3, 61.1, 60.0, 52.9, 50.8, and 37.0.

*Methyl (methyl 4-azido-3,4-dideoxy-*D-arabino-*heptulopyranosid)onate 7-(diphenyl phosphate)* (**13**).—Compound **12** (1.8 g, 6.93 mmol) was dissolved under Ar in pyridine (170 mL) stored over molecular sieves. Freshly distilled diphenylchlorophosphate (0.45 mL, 2.17 mmol) was added at room temperature. The pale yellow solution became brownish and was stirred for 14 h at room temperature. Additional diphenylchlorophosphate (1 mL, 4.83 mmol) was added and stirring was continued for 24 h, when further diphenylchlorophosphate (0.3 mL, 1.44 mmol, 8.44 mmol total) was added. After an additional 7 h the reaction was quenched with water (5 mL). The solvent was removed under high vacuum and the residue was dissolved in $CHCl_3$ (200 mL). The solution was washed three times with water (20 mL each) and the combined aqueous phases were reextracted twice with $CHCl_3$ (5–10 mL). The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The residue was subjected to chromatography (1:1 *n*-hexane–EtOAc) to give **13** (R_f 0.43) as a heavy syrup in quantitative yield (3.5 g, 7.06 mmol). HRFABMS: *m/z* 494.1309 $(M + H)^+$; calcd for $(C_{21}H_{24}O_9N_3P + H)$ 494.1328. ^1H NMR (300 MHz, $CDCl_3$): δ 7.32 (m, 4 H, Ph), 7.20 (m, 6 H, Ph), 4.57 (ddd, 1 H, $J_{H\text{-}7eq,P}$ 9.0, $J_{H\text{-}7eq,H\text{-}7ax}$ 11.8, $J_{H\text{-}7eq,H\text{-}6}$ 4.0 Hz, H-7*eq*); 4.42 (dt, 1 H, $J_{H\text{-}7ax,H\text{-}7eq}$ 11.9, $J_{H\text{-}7ax,H\text{-}6} = J_{H\text{-}7ax,P}$ 9.0 Hz, H-7*ax*), 3.77 (s and dd, 4 H, $J_{H\text{-}4,H\text{-}5}$ 9.8, $J_{H\text{-}4,H\text{-}3eq}$ 5.0 Hz, $COOCH_3$ and H-4), 3.60 (m, 1 H, H-6); 3.28 (t, 1 H, $J_{H\text{-}5,H\text{-}4} = J_{H\text{-}5,H\text{-}6} = 9.7$ Hz, H-5), 3.17 (s, 3 H, OCH_3), 2.26 (dd, 1 H, H_{3eq}, $J_{H\text{-}3eq,H\text{-}3ax}$ 13.2, $J_{H\text{-}3eq,H\text{-}4}$ 4.9 Hz, H-3*eq*), 1.50 (dd, 1 H, $J_{H\text{-}3ax,H\text{-}3eq}$ 13.1, $J_{H\text{-}3ax,H\text{-}4}$ 12.3 Hz, H-3*ax*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.7, 150.3, 150.2, 150.1, 129.8, 125.7, 125.5, 120.5, 120.4, 120.1, 120.0, 98.2, 72.9 (d, $J_{C\text{-}6,P}$ 3.1 Hz, C-6), 69.0, 67.2 (d, $J_{C\text{-}7,P}$ 5.5 Hz, C-7), 59.2, 52.6, 50.8, and 37.0.

*Methyl (methyl 4-amino-3,4-dideoxy-*D-arabino-*heptulopyranosid)onate 7-phos-*

phate (**14**).—Compound **13** (3.3 g, 6.73 mmol) was dissolved in MeOH (250 mL) and deionized water (10 mL) was added. The flask was flushed with Ar and platinum oxide (150 mg) was added. The flask was then flushed with H_2 and the solution was stirred for 24 h. An abundant white precipitate formed. The flask was flushed with Ar and deionized water was added until the precipitate was almost completely dissolved (25 mL). More platinum oxide was added (100 mg), the flask was flushed again with H_2, and the mixture was stirred overnight, forming more precipitate. Deionized water (45 mL) was added to dissolve the precipitate and the solution was filtered through Celite which had been washed with water. The filtrate was concentrated in a rotary evaporator and subsequently dried under high vacuum to give white crystals (2.3 g). At this point the ^{1}H NMR spectrum revealed incomplete removal of the phenyl groups. The hydrogenation was therefore repeated for 1 day to give, after a similar work-up, **14** (2.2 g, 100%) which was 95% pure as judged by NMR. ^{1}H NMR (300 MHz, D_2O): δ 4.10–3.97 (m, 2 H, H-7 and H-7′), 3.72 (s, 3 H, $COOCH_3$), 3.64 (m, 2 H, H-5 and H-6), 3.51 (br ddd, 1 H, H-4), 3.13 (s, 3 H, OCH_3), 2.35 (dd, 1 H, $J_{H\text{-}3eq,H\text{-}3ax}$ 13.3, $J_{H\text{-}3eq,H\text{-}4}$ 4.5 Hz, H-3*eq*), 1.88 (t, 1 H, $J_{H\text{-}3ax,H\text{-}3eq} = J_{H\text{-}3ax,H\text{-}4} = 12.8$ Hz, H-3*ax*). ^{13}C NMR (75 MHz, D_2O): δ 167.3, 95.9, 71.2 (d, $J_{C\text{-}7,P}$ 7.7 Hz, C-7), 63.7, 61.2 (d, $J_{C\text{-}6,P}$ 3.3 Hz, C-6) 51.8, 49.1, 47.6, 33.1. ^{31}P NMR (121.5 MHz, D_2O): δ 1.98 (t, J 6.2 Hz).

4-Amino-3,4-dideoxy-D-arabino-*heptulosonic acid 7-phosphate* (**15**).—Deprotection was first attempted by dissolving **14** (2.2 g) in freshly prepared 6 N HCl (160 mL) and heating the solution to 50°C for 2 days. However, the NMR spectrum of the crude mixture showed incomplete deprotection of the anomeric alcohol function although the ester function was completely removed. The compound was then dissolved in freshly prepared 6.5 N HBr (160 mL) and the solution was stirred for 2 days at 50°C. The mixture was concentrated under vacuum at a temperature not exceeding 35°C. Water was repeatedly added to the residue and evaporated until no more fumes were observed upon addition of water. The residue (1.8 g, 90% yield) solidified upon prolonged drying under high vacuum, but the material is extremely hygroscopic. Extensive efforts to recrystallize the product were unsuccessful. The compound decomposed in the range 115–120°C with evolution of gas. The purity of the final material was estimated by NMR as 93–95%. $[\alpha]\ \Theta_{241}$ −0.9°, Θ_{220} +0.2°, Θ_{208} −0.05° (*c*, 10 mM, H_2O, 22°C). HRFABMS: *m*/*z* 288.0501, $(M+1)^+$; calcd for $(C_7H_{14}NO_9P + H)$ 288.0484. ^{1}H NMR (300 MHz, D_2O): δ 4.09 (m, 2 H, H-7,7′), 3.91 (br d, 1 H, H-6), 3.62 (t, 1 H, $J_{H\text{-}5,H\text{-}6} = J_{H\text{-}5,H\text{-}4} = 9.7$ Hz, H-5), 3.54 (ddd, 1 H, H-4), 2.26 (dd, 1 H, H_{3eq}, $J_{H\text{-}3eq,H\text{-}4}$ 4.1 Hz, H-3*eq*), 1.95 (t, 1 H, $J_{H\text{-}3ax,H\text{-}3eq} = J_{H\text{-}3ax,H\text{-}4} = 12.5$ Hz, H-3*ax*). ^{13}C NMR (75 MHz, D_2O): δ 169.0, 91.2, 69.7 (d, $J_{C\text{-}7,P}$ 7.4 Hz, C-7), 63.3, 61.7 (d, $J_{C\text{-}6,P}$ 4.63 Hz, C-6), 47.0, 31.8. ^{31}P NMR (121.5 MHz, D_2O): δ 1.38.

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