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Synthesis of Methyl 1-(2,3,5-Tri-*O*-acetyl-β-L-ribofuranosyl)-1,2,4-triazole-3-carboxylate from L-Ribose: From a Laboratory Procedure to a Manufacturing Process

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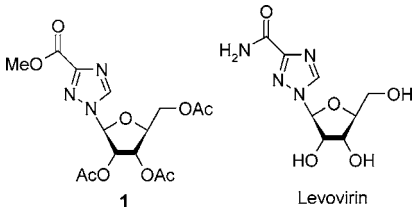
Abstract:

A two-step manufacturing process for methyl 1-(2,3,5-tri-*O*-acetyl-β-L-ribofuranosyl)-1,2,4-triazole-3-carboxylate (1) was developed. In step 1, L-ribose was converted to a β/α mixture of 1,2,3,5-tetra-*O*-acetyl-L-ribofuranoses (2 and 4). The step contained four chemical transformations and was completed in "one-pot" in approximately 95% yield. The crude step 1 product was reacted with methyl 1,2,4-triazole-3-carboxylate (3) in step 2 to produce 1. The successful utilization of both isomers (2 and 4) in step 2 offered advantages of higher overall yield and a much simplified process by eliminating the isolation of pure 2. The process was successfully scaled up to the pilot plant and subsequently in a manufacturing campaign using commercial production facilities.

Introduction

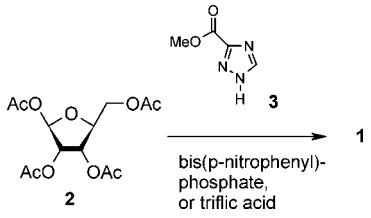
Methyl 1-(2,3,5-tri-*O*-acetyl-β-L-ribofuranosyl)-1,2,4-triazole-3-carboxylate (**1**) was an intermediate for the synthesis of Levovirin,[1] which was developed as an antiviral agent to treat hepatitis C. In the early kilo-lab campaigns, **1** was



prepared from 1,2,3,5-tetra-*O*-acetyl-β-L-ribofuranose (**2**), as illustrated in Scheme 1. Commercially available compound **2** was very expensive; therefore the cost of goods became a serious issue for the commercial success of the drug. To overcome this problem, we decided to introduce an in-house production of **2** from considerably less expensive L-ribose.

Only one literature procedure was found for the conversion of L-ribose to compound **2**.[2] The procedure described

Scheme 1. **Kilo-lab synthesis of 1**



in the literature was tedious, containing 18 extractions and 8 distill-to-dryness operations, and was deemed not suitable for scale-up. Part of the reason for the process being cumbersome was the complex nature of the conversion. In most solvents ribose exists as an equilibrium mixture of five isomers: one acyclic form, two ribofuranoses, and two ribopyranoses (Scheme 2, R = H).[3] In early studies, direct acetylation of ribose under different conditions formed a mixture of up to five products, with the ribopyranoses being the major component in most cases.[4] Therefore, to achieve an efficient conversion of ribose to ribofuranoses, a stepwise approach had to be adopted. It was reported[5] that methanolyses of most of the pentoses under acidic conditions afforded the kinetically favored methyl furanosides first, and then the furanosides gradually converted to the thermodynamically favored pyranosides until an equilibrium was reached (Scheme 2, R = CH_3). If the methanolysis was conducted under mild conditions and the equilibrium was stopped before significant amounts of the pyranosides were formed, the furanosides could be obtained as the major products. Based on this approach, a strategy to convert D-ribose to 1,2,3,5-tetra-*O*-acetyl-β/α-D-ribofuranoses was developed by Guthrie et al.[6] As indicated in Scheme 3, D-ribose was first converted to methyl ribofuranosides (**methanolysis**) in methanol with a catalytic amount of strong acid. The ribofuranosides were then converted to methyl 2,3,5-tri-*O*-acetyl-β/α-D-ribofuranosides (**acetylation**) by reacting with acetic anhydride under basic conditions, followed by **acetolysis** in acetic acid and acetic anhydride to afford 1,2,3,5-tetra-*O*-acetyl-β/α-D-ribofuranoses. The

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(2) (a) Ramasamy, K. S.; Tam, R. C.; Bard, J.; Averett, D. R. *J. Med. Chem.* **2000**, *43*, 1019. (b) Ramasamy, K. S.; Hills, L.; Tam, R.; Averett, D. U.S. Patent 6,130,326, 2000.

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(6) Guthrie, R. D.; Smith, S. C. *Chem. Ind. (London)* **1968**, 547.

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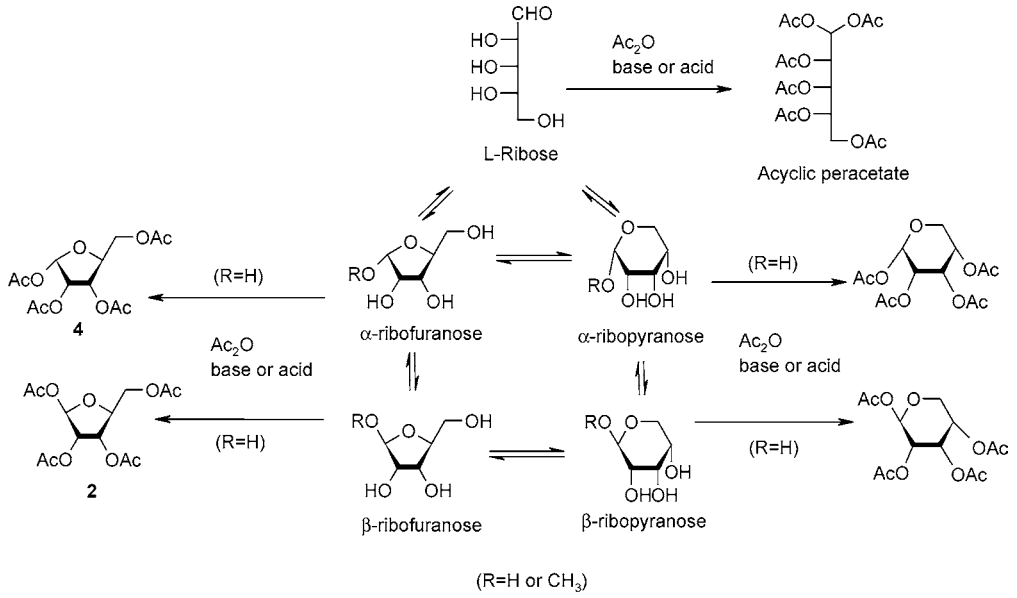
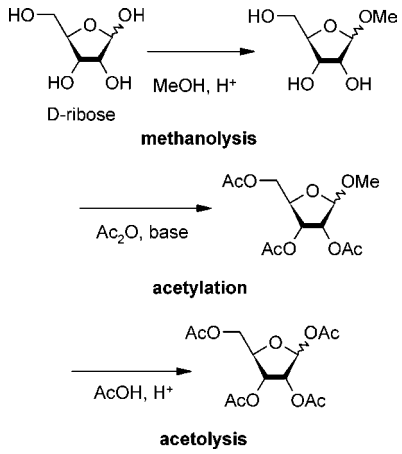
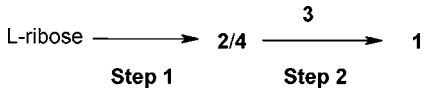
Scheme 2. **Possible products of direct acetylation of L-ribose and methanolysis under acidic conditions**



Scheme 3. **Guthrie's method of converting D-ribose to tetra-*O*-acetylribofuranoses**



Scheme 4. **Overall development plan**

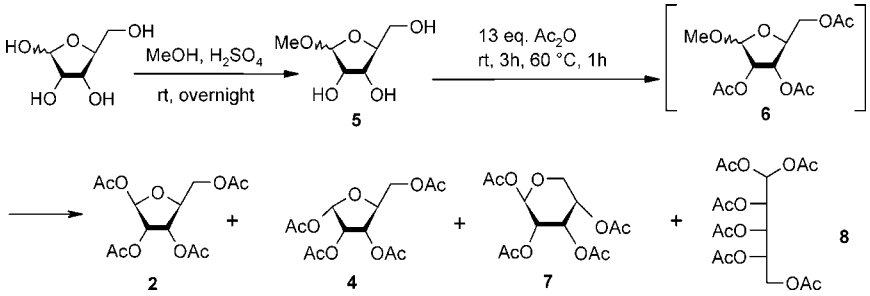


same strategy was used in the literature synthesis of **2**,[2] in which the methanolysis was carried out in methanol and HCl, the acetylation was conducted in acetic anhydride and pyridine, and the acetolysis was carried out in a mixture of acetic anhydride and concentrated sulfuric acid in acetic acid to afford a mixture of **2** and its α-anomer, **4**. Finally, pure **2** was isolated in 57% yield via recrystallization from ethyl ether.

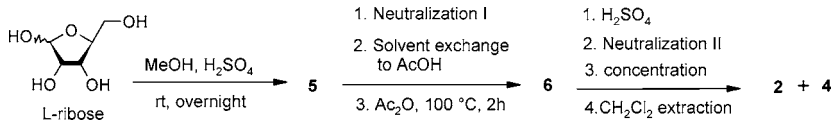
Our development plan for the project included (1) developing a more efficient process for the conversion of L-ribose to **2**/**4** (Scheme 4, Step 1) starting from the modification of the literature procedure and (2) exploring the possibility of converting both **2** and **4** to **1** (Step 2), so as to avoid isolating pure **2** and to improve the overall yield.

Establishment of the Basic Process for Step 1. The key to streamlining the literature process was to eliminate the unnecessary solvent exchanges and the isolation of intermediates so that the number of extractions and distillations could be significantly reduced. We envisaged that if the acetylation could be carried out under acidic conditions the overall conversion could be done in "one-pot". For instance, the methanolysis could be conducted in methanol using sulfuric acid as catalyst. Upon completion of the reaction the mixture would contain methyl *β*/α-L-ribofuranosides (**5**), sulfuric acid, methanol, and water. Addition of an excess amount of acetic anhydride to the mixture would lead to the formation of methyl 2,3,5-tri-*O*-acetyl-*β*/α-L-ribofuranosides (**6**) in a medium containing sulfuric aid, acetic acid, and unreacted acetic anhydride, favorable conditions for acetolysis. The reaction could then be continued until the completion of acetolysis. An example is shown in Scheme 5, in which L-ribose was stirred with sulfuric acid in methanol at room temperature overnight. To this mixture was slowly added 13 equiv of acetic anhydride. The mixture was then stirred at room temperature for 3 h and 60 °C for 1 h. The reaction generated a crude product containing 20% **2**, 13% **4**, 16% 1,2,3,4-tetra-*O*-acetyl-*β*-L-ribopyranose (**7**), and 33% acyclic peracetate (**8**). Compound **7** could be formed via several different pathways; however two conditions had to exist: one was the opening of the five-member ring (**5** and/or **6**), and the other was the presence of a free 5-hydroxy group. The formation of **8** also required the ring opening of the ribofuranoses. To minimize the formation of the two byproducts, it was necessary to carry out the overall conversion under mild conditions (to prevent the ring opening) and to acetylate the 5-hydroxy group before conducting acetolysis. Based on this analysis several changes were made. First, upon the completion of methanolysis, sodium acetate was added to neutralize sulfuric acid and stop the acid-catalyzed equilibria. Second, after neutralization the mixture was concentrated to partially remove methanol. Acetic anhydride was then added at ambient temperature, and the mixture was heated at 100 °C for 2 h to complete the acetylation. Third, after the mixture was stirred with sulfuric acid at room temperature for 1 h, it was neutralized with sodium acetate.

Scheme 5. **A "one-pot" acetylation of L-ribose**



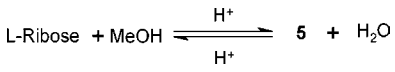
Scheme 6. **New process for step 1**



This reaction mixture was then concentrated and extracted with dichloromethane to afford a crude product. This time the crude product contained 66% **2**, 24% **4**, 1.3% **7**, and 2.8% **8**. The overall yield was 88%. Although the results were encouraging, there was a safety concern in the acetylation step. The reaction of methanol and the hydroxyl groups in **5** with acetic anhydride are strongly exothermic.[7] After the addition of acetic anhydride to the mixture at ambient temperature, a gradual increase in batch temperature was observed. The temperature increase accelerated quickly, and strong external cooling had to be applied to keep the reaction under control even on small scale batches in the lab. Two measures were taken to address this problem. One was to completely remove methanol from the system by solvent-exchange to acetic acid before the addition of acetic anhydride. The other was to slowly add acetic anhydride at high temperature (e.g., 50−80 °C) so that there would be no acetic anhydride accumulation in the system. After these modifications a basic framework of the process had been established, which is illustrated in Scheme 6. This version of the process contained only one solvent-exchange operation, one concentration, and one extraction.

Optimization of Step 1. After the establishment of the basic process for the conversion, the step was further optimized. The methanolysis was carried out by stirring L-ribose and methanol at ambient temperature in the presence a of catalytic amount of concentrated sulfuric acid. The reaction was an equilibrium between L-ribose/methanol and **5**/water. With 0.1−0.2 equiv of sulfuric acid, the reaction reached its equilibrium within 2 h. Experimental data indicated that 20 ± 5 °C was the ideal temperature for the reaction. At higher temperature the formation of methyl ribopyranosides became significant. At lower temperature it took a longer time for the reaction to reach equilibrium while no improvement of conversion and stereoselectivity was observed. The most effective way to push the equilibrium towards the product was to use more methanol. As demonstrated in the examples in Table 1, when the methanol was increased from 2 mL/g of L-ribose to 15 mL/g of L-ribose, the percentage of product in the reaction mixture

Table 1. **Concentration effect on the methanolysis**



entry	mL of MeOH/ g of L-ribose	GC% (area) **5**	GC% (area) L-ribose
1	2	91.5−93.4	4.5−5.1
2	5	95.8−97.6	1.8−2.5
3	9	97.2−97.8	1.7−1.9
4	15	98.7	1.4

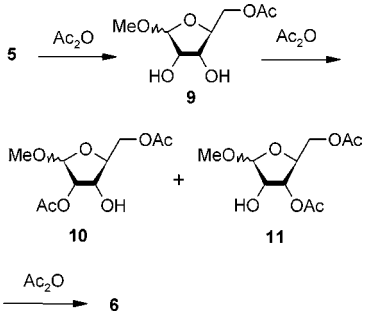
was improved from 92 to 93% to 99%. Ultimately, a concentration of 5 mL of methanol/g of L-ribose was adopted as a compromise of conversion and process capacity.

After the methanolysis reached its equilibrium, a base was added to bring the pH of the mixture to above 4, at which the equilibrium was halted. Several bases were evaluated for this purpose. For the neutralization per se any base could be used, however the choice of base could affect the subsequent transformations and operations. For instance, when sodium acetate was used the subsequent acetylation and acetolysis went normally. The byproduct (Na_2SO_4), however, has very low water solubility (4.8 g/100 mL[8]) which required the use of large quantities of water during the extraction and resulted in significant loss of process capacity. Ammonium sulfate has very good water solubility (71 g/100 mL[8]). However, when NH_4OAc was tested for the neutralization an intractable mixture was produced during the acetylation. When pyridine was tested the acetylation went faster and the acetolysis was normal. Unfortunately, significant product (**2**/**4**) decomposition was observed during the final concentration of the reaction mixture. Lithium sulfate has a water solubility of about 26 g/100 mL,[8] so both LiOAc and Li_2CO_3 were tested for the neutralization. No adverse effects on the process were observed in both cases. Lithium carbonate was chosen based on cost consideration. It is worth noting that the particle size of Li_2CO_3 was crucial for the neutralization. Fine powder must be used. When granular Li_2CO_3 was tested in some of lab batches, an intractable mixture was formed after acety-

(7) Reaction calorimetry data measured in our Risk Analysis Lab: $\Delta H_{reaction} = -173$ kJ/mol, $\Delta T_{ad-rise} = 71$ °C.

(8) *Handbook of Chemistry and Physics*, 70th ed.; Section B, Physical Constants of Inorganic Compounds; CRC Press.

Scheme 7. **Details on the acetylation reaction**



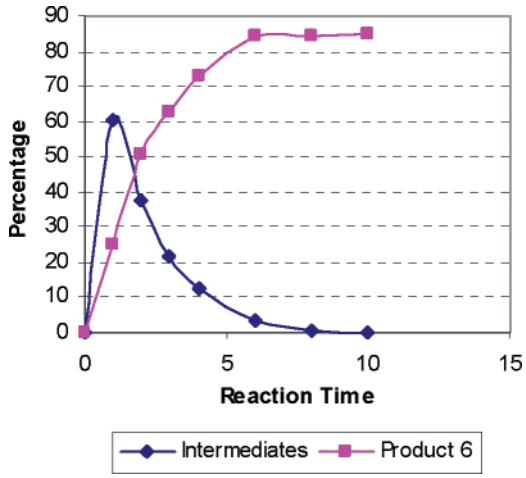


Figure 1. **Kinetics of acetylation at 60 °C.**

lation. It was possible that the large particle size caused inadequate neutralization, which in turn resulted in the reverse of **5** to L-ribose during the solvent exchange.

During the acetylation compound **5** was converted to **6**. There are three different hydroxyl groups in **5**. The acetylation should occur in a step-by-step fashion via mono- and diacetylated intermediates (**9**, **10**, and **11** in Scheme 7). Monoacetylation, presumably at the primary hydroxyl group, was fast. For instance, when treated with acetic anhydride at 50 °C, **5** disappeared completely within 1 h, leading to the formation of a mixture of **9**, **10**, **11**, and **6**. The conversion of **9**−**11** to **6** was slower and required a higher temperature and longer reaction time. Figure 1 illustrates a typical reaction profile for the acetylation at 60 °C. At the beginning of the reaction the mixture contained mainly the intermediates **9**−**11**. As the reaction proceeded the intermediates were gradually converted to the product. Based on this observation the reaction rate was measured as the consumption of the total intermediates (the sum of **9**, **10**, and **11**).

The rate of the acetylation was affected by both reaction temperature and the amount of acetic anhydride. As indicated in Figure 2, when 5 equiv of acetic anhydride was charged, the intermediate level dropped to 0.76% in 8 h and totally disappeared in 10 h at 60 °C. At 80 °C, the reaction took less than 4 h. When the temperature was increased to 100 °C, all the intermediates disappeared within 1 h, and no deterioration of product purity was observed. Based on the results, 100 °C was adopted for the process.

The effect of acetic anhydride on the acetylation at 100 °C was demonstrated in Figure 3. When 5 equiv of acetic

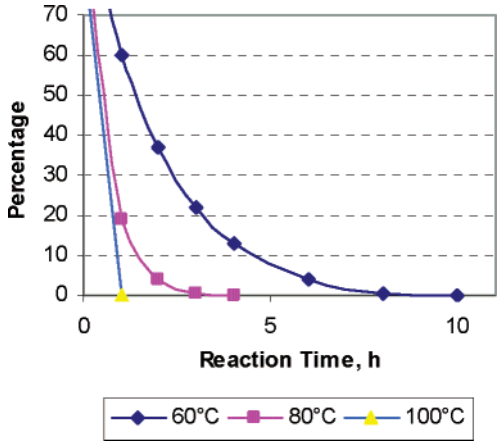


Figure 2. **Temperature effects on Acetylation.**

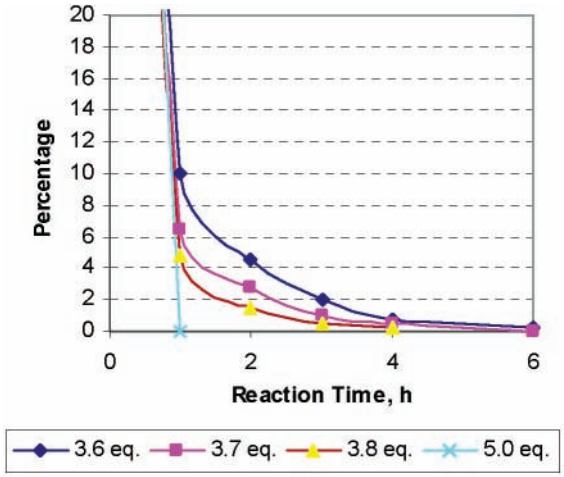


Figure 3. **Effect of acetic anhydride charge on acetylation at 100 °C.**

anhydride were charged, acetylation was completed within 1 h. When 3.8 equiv of acetic anhydride was added, the reaction was much slower. It took about 4 h for the intermediates to drop to 0.27%. Reducing the acetic anhydride charge to 3.7 equiv made the reaction even slower. It took 4 h for the intermediate levels to drop to 0.59%, and the reaction was completed (total consumption of the intermediates) in 6 h. When 3.6 equiv of acetic anhydride were charged, the reaction was not complete after 6 h. As will be discussed later, to reduce the impurity level during the acetolysis, it was necessary to tightly control the amount of acetic anhydride in the reaction mixture. As a compromise, 3.7 equiv of acetic anhydride was charged for the acetylation process.

Upon the completion of acetylation the reaction mixture was cooled and treated with sulfuric acid to effect acetolysis. With the isolation of pure **2** in mind, our initial focus was how to increase the ratio of **2** to **4** in the reaction. It was discovered that the ratio was temperature dependent and was not affected by the β/α ratio of **6**. Figure 4 illustrates the ratio changes of β/α-**6** and **2/4** during acetolysis at 0 °C. The β/α ratio of **6** increased sharply at the beginning of the reaction and then remained constant. The ratio of **2** to **4** was around 7 and decreased steadily to about 3 in 6 h. The following conclusions could be drawn from the observa-

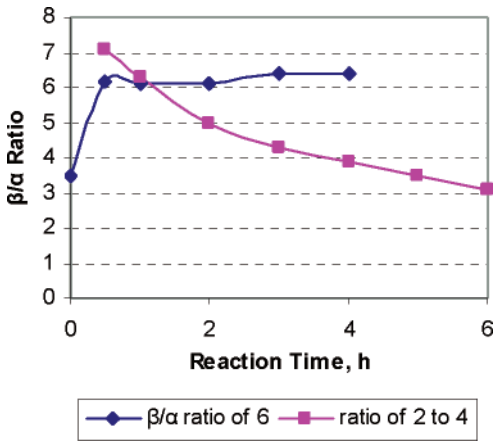
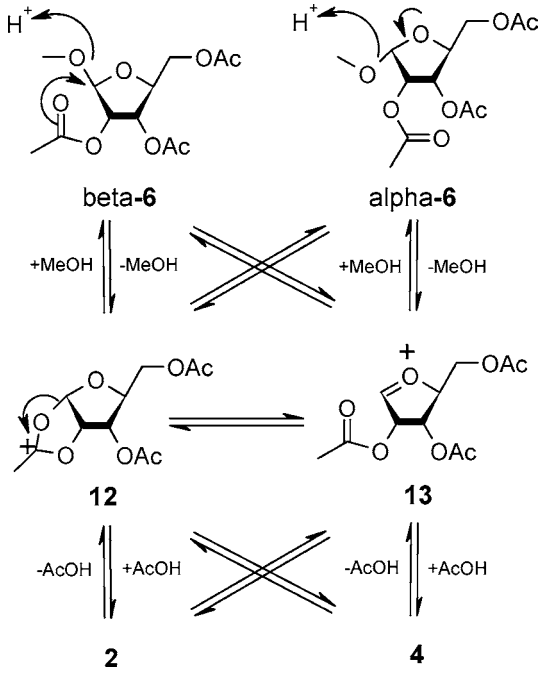


Figure 4. **β/α ratio changes during acetolysis.**

Scheme 8. **Proposed mechanism for acetolysis**



tions: (1) α-**6** was more reactive than *β*-**6**. The fast consumption of α-**6** at the beginning of the reaction caused the increase of the *β*/α ratio; (2) **2** was formed faster than **4**; (3) there was an equilibrium between **2** and **4**, and part of the newly formed **2** was gradually epimerized to **4**. Based on these observations, a mechanism for the acetolysis reaction was proposed and is shown in Scheme 8. Multiple equilibria existed in the reaction mixture, including the equilibrium between the two anomers of **6**, the equilibrium between **2** and **4**, and the equilibrium between **6** and **2/4**. The driving force of the reaction was the consumption of methanol, which was released from **6** during the reaction, by acetic anhydride. Both *β*/α-**6** passed through the same intermediates, **12** and **13**, to form **2/4**. As a result, the *β*/α ratio of **6** should not have any effect on the ratio of **2** to **4**. The ratio of **2** to **4** should be determined by the equilibrium between the two isomers. Therefore, the major factors that would most likely affect the **2/4** ratio would be the reaction temperature and reaction medium. The amount of the sulfuric acid should affect only the reaction rate, but not the **2/4** ratio.

Table 2. **Effects of temperature and sulfuric acid on acetolysis**

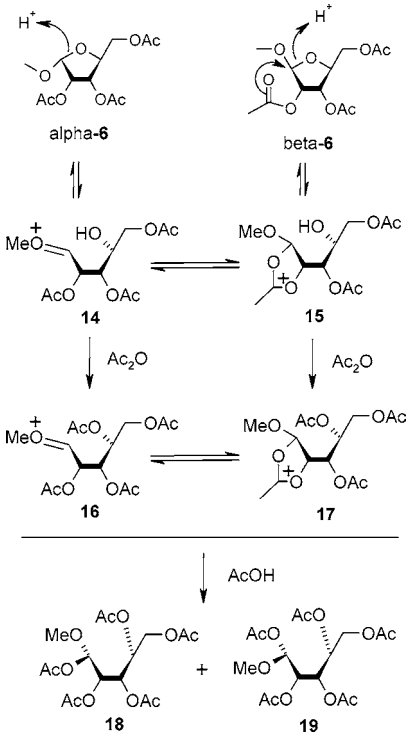
entry	*T*, °C	equiv of H_2SO_4	reaction time, h	GC% **2**	GC% **4**	GC% **2** + **4**
1	0	0.97	0.5	67	23	90
2	0	0.82	1	67	23	90
3	0	0.43	6	66	22	88
4	−13	0.82	2	71	17	88
5	−23	0.82	4	66	13	79

These hypotheses were confirmed in our lab, and some results are listed in Table 2. For instance, the effect of sulfuric acid on the reaction at 0 °C is demonstrated in entries 1 to 3. With 0.97 equiv of sulfuric acid, the reaction was completed within 0.5 h and the system reached equilibrium (ratio of **2** to **4** became constant) within 0.5 h to give about 67% of **2** and 23% of **4**. With 0.82 equiv of sulfuric acid, the reaction completed within 0.5 h but reached equilibrium in 1 h. When 0.43 equiv of sulfuric acid was used, the reaction completed in about 5 h and the system reached equilibrium in about 6 h. The products from all the three batches had the same composition and purity profiles. Based on these results, 0.8 equiv of sulfuric acid was adopted for the process.

Reaction temperature affected both reaction rate and *β*/α ratio of the products. For instance, in the presence of 0.82 equiv of sulfuric acid, the reaction was completed in 1 h at 0 °C and the **2/4** ratio was 76/23 (entry 2). At −13 °C the reaction was completed in about 2 h and the **2/4** ratio reached 71/17 (entry 4). At −23 °C the reaction took 4 h and the **2/4** ratio was increased to 66/13 (entry 5). However, at −23 °C higher amounts of impurities were formed which affected the yield. Clearly, if pure **2** is to be isolated the acetolysis should be carried out at low temperature (e.g., −13 °C). However, if both isomers are to be utilized in the subsequent steps, lower reaction temperature would not offer any benefit.

Using the optimized conditions up to this point the process produced a crude product that contained about 90% (GC area%) of **2/4**. Two major impurities with close retention times on GC accounted for most of the other 10%. Experimental data indicated that (1) the impurity level was proportional to the amount of acetic anhydride added to the reaction mixture, as higher levels of the impurities were observed when more acetic anhydride was used; (2) the amount of sulfuric acid had no effect on the impurity level; (3) when pure **2** was subjected to the acetolysis condition the product was a mixture of **2** and **4**, with none of the impurities observed in the reaction mixture; and (4) when pure *β*-**6** was subjected to the acetolysis condition the reaction mixture contained not only **2** and **4** but also the two impurities. These results clearly indicated that the impurities were formed from **6** during the acetolysis via a competing mechanism. As indicated in Scheme 8, the conversion of **6** to **2/4** started with the protonation of the methoxy group, followed by the leaving of a molecule of methanol to give intermediates **12** and **13**. A competing mechanism would be the protonation of the oxygen in the five-member ring, followed by the ring opening to give intermediates **14** and **15** (Scheme 9). Acetylation of the hydroxyl group in the

Scheme 9. **Possible mechanism for the impurity formation**



presence of acetic anhydride would lead to the formation of **16** and **17**. Addition of acetic acid to **16** and **17**, followed by deprotonation would give compound **18** and **19**. To support this hypothesis, the two impurities were isolated and identified by spectroscopy as **18** and **19**. To further confirm the structures, the two compounds were independently synthesized using a literature procedure.[9]

After identification of the two impurities, the remaining challenge was to minimize their formation. According to the mechanism in Scheme 9, the driving force for the formation of **18** and **19** was the acetylation of the 4-hydroxyl group after the ring opening. Apparently, the presence of a high concentration of acetic anhydride favored this path, which was consistent with our observations. Lowering the acetic anhydride concentration during the acetolysis seemed to be a logical approach. In our "standard" procedure, the total amount of acetic anhydride charged for both acetylation and acetolysis was 5.5 equiv and the anhydride was added in one portion. We found that the charge could be reduced to 5.0 equiv without affecting the reaction. However, the reduction did not lead to a significant decrease of the impurity level. To keep the concentration of acetic anhydride low during the acetolysis, it was decided to add acetic anhydride in two portions. In the first portion, enough anhydride (3.7 equiv) was added to effect the acetylation. Upon the completion of the acetylation, sulfuric acid was added, followed by the addition of the second portion of the anhydride (1.4 equiv). Studies indicated that the addition mode had an affect on the impurity level (Table 3). With a total amount of 5.1 equiv of acetic anhydride used, the process using a one portion addition generated 6.9% of total

Table 3. **Impurity level changes under different conditions**[a]

entry	1st portion of Ac_2O (equiv)	2nd portion of Ac_2O (equiv)	addition temp (°C)	addition time	GC% area **18** + **19**
1	5.1	0	5[b]	NA	6.9
2	3.7	1.4	5	5 min	5.7
3	3.7	1.4	5	1 h	3.7
4	3.7	1.4	5	2 h	3.0
5	3.7	1.4	5	3 h	2.9
6	3.7	1.4	20	2 h	0.7
7	3.7	1.4	25	2 h	0.4

[a] All the batches were carried out on a scale of 66.6 mmol of L-ribose. The first portion of Ac_2O was added at 80 °C. The mixture was heated to 100 °C, held for 4 h, and then cooled to 5 °C. Sulfuric acid (0.82 equiv) was added, followed by the addition of the second portion of Ac_2O at the designated temperature in a period of time as indicated in the table. [b] The temperature at which sulfuric acid was added.

impurities. When 3.7 equiv of the anhydride was added for the acetylation and then another 1.4 equiv was added in 5 min following the sulfuric acid charge, the impurity level dropped to 5.7% (entry 2). It was interesting to note that the addition rate of the second portion of the anhydride affected the impurity level significantly. When the 1.4 equiv of acetic anhydride was added slowly in 1 to 3 h, the impurity level could be reduced to 2.9% (entries 3 to 5). It was also discovered that the reaction temperature affected the impurity level. As indicated in entries 5 and 6, the impurity level decreased to 0.7% from 2.9% when the reaction temperature was changed from 5 °C to 20 °C. Further increase of the temperature to 25 °C resulted in lower overall yield due to higher levels of other impurities. Based on these results, the second portion of acetic anhydride was added slowly at 20 °C over at least 2 h in the process.

Upon the completion of the acetolysis the reaction mixture was transferred to a lithium carbonate solution. The mixture was then concentrated and extracted. Several solvents (dichloromethane, ethyl acetate, α,α,α-trifluorotoluene, and toluene) were evaluated for this purpose, and dichloromethane gave the best results. Two dichloromethane extractions were adopted in the process, so as to efficiently extract the product without sacrificing the process capacity. *After optimization of the step 1 process, the step 1 product (a mixture of **2** and **4**) could be obtained from L-ribose in approximately 95% overall yield.*

Establishment of the Step 2 Process and Its Optimization. Two methods for the fusion of **2** with methyl 1,2,4-triazole-5-carboxylate (**3**) (Scheme 1) were used in the early kilo-lab campaigns. One was the ICN process[2] in which the reaction was carried out at 165−175 °C using bis(*p*-nitrophenyl)phosphate as catalyst. The other was a process developed in our Process Research lab for the production of Ribavirin[10] in which the reaction was conducted at 120−130 °C in the presence of triflic acid as catalyst and the product was isolated via crystallization from methanol. In both processes only pure **2** was used. As mentioned above, the current step 1 process produced a mixture of **2** and **4** in

(9) Lichtenthaler, F. D.; Breunig, J.; Fischer, W. *Tetrahedron Lett.* **1971**, *12*, 2825.

(10) Ribavirin is a drug currently in the market for the treatment of hepatitis C and is the enantiomer of Levovirin. For the synthesis of Ribavirin, see: Witkowski, J. T.; Robin, R. K.; Sidwell, R. W.; Simon, L. N. *J. Med. Chem.* **1972**, *15*, 1150.

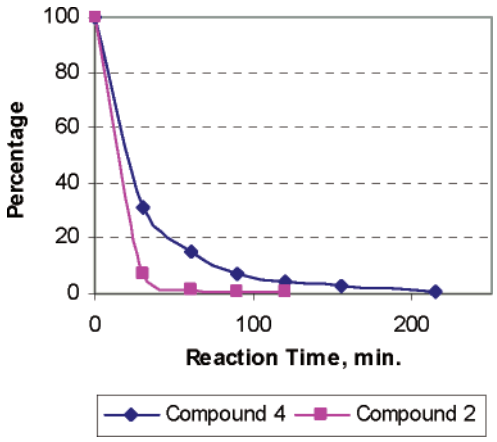


Figure 5. **Reaction rate of 2 and 4 as the consumption of 3.**

a ratio of approximately 3:1. The use of pure **2** in the step 2 reaction would mean the loss of about 25% of the expensive step 1 product and additional purification operations for the isolation of pure **2**. It would be a significant advantage in terms of production cost and efficiency if the mixture of both anomers could be converted to the desired product.

First, the reaction of pure **2** with **3** was tested using the Ribavirin process. Thus, a mixture of **2**, 1.0 equiv of **3**, and a catalytic amount of triflic acid (1% mol) in methyl acetate was concentrated and heated at 120−130 °C for approximately 5 h under vacuum to complete the reaction. The mixture was then cooled to 70 °C, and methanol was added. The mixture was stirred until a homogeneous solution was formed and then slowly cooled to 0 °C to precipitate **1** in 75% yield. To prove the concept that **4** could also be converted to **1**, a pure sample of **4** was isolated from a batch of Step 1 product. The sample was treated with **3** and triflic acid under the same condition as above. *The reaction proceeded normally, and product **1** was isolated in 70% yield!* The only difference observed for the two compounds was that **2** reacted faster than **4**, as illustrated in Figure 5.

Having confirmed that both **2** and **4** could be utilized in Step 2, the crude Step 1 product was tested. When the crude product was subjected to the same process conditions the results were not consistent. In the experiments, compound **3** was added to the step 1 dichloromethane extract. The mixture was concentrated to dryness, and then triflic acid was added to start the reaction. The main problem was the difficulty in initiating the reaction. In some cases much higher triflic acid charges (up to 6 mol %) were needed to complete the reaction. The use of a high level of triflic acid led to significant yield loss and colorization of the product. It seemed that there were some basic materials in the crude step 1 solution that were consuming triflic acid. To solve this problem, it was decided to wash the dichloromethane solution of crude step 1 product with dilute acid (e.g., 5% sulfuric acid) before starting step 2. Indeed, after the acid wash the step 2 reaction could be completed within 5 h under the same conditions in the presence of as low as 1 mol % of triflic acid.

In the early stage of the development it was found that one source of yield loss in step 2 was the hydrolysis of **1** due to the presence of water in the reaction system. We have detected seven impurities derived from the hydrolysis of **1**: three "monohydrolyzed" (compounds with one of the three acetyl groups in **1** removed), three "dihydrolyzed" (compounds with two of the three acetyl groups removed), and one "trihydrolyzed" product. Due to high solubility of these impurities in methanol, they stayed in the mother liquor during the crystallization. In some cases these impurities accounted for up to 7% of yield losses. Therefore, drying the dichloromethane solution of Step 1 product became necessary. Some drying agents, such as sodium sulfate or magnesium sulfate, could be used to dry the solution. While adding and filtering the drying powder was not a problem in the lab, it did raise concerns on the prospect of large-scale production where handling large amounts of solids might be problematic and disposal of large quantities of solid wastes could be costly. Also, 1.5−3.5% of hydrolyzed products were formed even after the step 1 solution was dried with sodium sulfate or magnesium sulfate. Therefore, we decided to evaluate other drying methods. One option was azeotropic distillation using toluene, but this would add extra operations to the process. Another option was to use a water-scavenger to consume the water in situ, such as acetic anhydride which would react with water to form acetic acid. The acetic acid and the unreacted anhydride could then be removed during the concentration step and before adding triflic acid. One concern for this approach was the possibility of the reaction of **3** with acetic anhydride. Thus, a mixture of acetic anhydride and **3** in acetic acid was stirred at 57 °C for 4 h, 85 °C for 1 h, and 115 °C for 2 h, and no reaction was observed. After confirming that there was no cross reaction between acetic anhydride and **3**, the effect of acetic anhydride on step 2 was studied. Some results are listed in Table 4. In the experiments, a batch of step 1 solution was divided into three portions. Each portion was mixed with 1.0 equiv of **3**, and then two of the batches were treated with 0.9 equiv and 2.6 equiv of acetic anhydride. All the batches were carried through step 2. The total percentage of the hydrolyzed step 2 products and the isolated yields were compared, and the benefits of adding acetic anhydride were obvious. For batches with added acetic anhydride (entry 2 and 3), the reactions were faster, the levels of hydrolyzed step 2 product were lower, and the yield was higher.

Table 4. **Effect of acetic anhydride charge on step 2**

entry	Ac_2O added (equiv)	% of hydrolyzed step 2 product	yield of isolated product (%)
1	0	2.66	79
2	0.9[a]	1.12	81
3	2.6[a]	0.69	86

[a] Relative to L-ribose.

Ideally, the amount of acetic anhydride charged should be just enough to consume all the water in the mixture. This was difficult to achieve due to the fact that the water content in the Step 1 extract varied from batch to batch. Excess anhydride could be added; however the unreacted anhydride

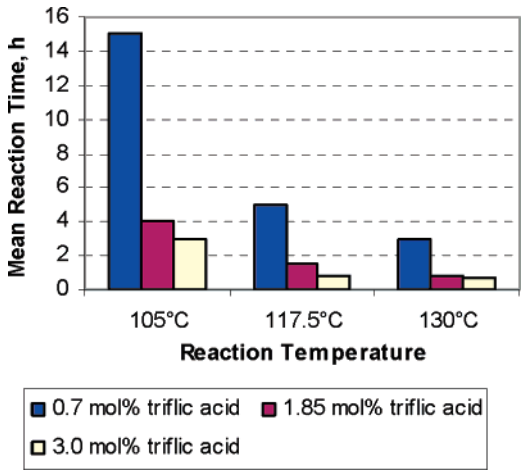


Figure 6. **Effects of temperature and triflic acid on the reaction rate.**

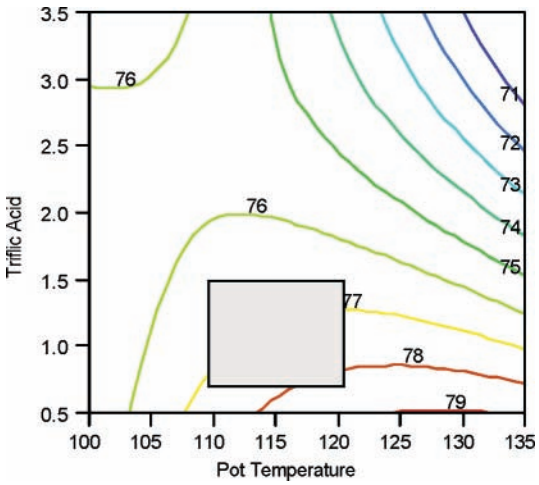


Figure 7. **Yield change and ideal parameter window for step 2.**

must be distilled out as completely as possible before charging triflic acid. Experimental data from additional studies demonstrated that 37 g (or 0.56 equiv) of acetic anhydride per batch of 100 g of L-ribose provided the best results.

Due to the fact that the Step 1 product was not isolated, the total amount of **2** and **4** in the step 1 extract was estimated via wt % assay of the solution. The amount of **3** charged in step 2 was then calculated based on the wt % assay. Ideally, the amount charged should be enough to convert all **2** and **4** to product **1**, but not so large an excess as to contaminate the product with unreacted **3**. Our experimental data indicated that 1.0−1.05 equiv (relative to total amount of **2** and **4** calculated from the wt % assay) of **3** were ideal for the process. Because the overall yield for step 1 was about 95%, the amount of **3** added was about 0.95 equiv of L-ribose. During the Manufacturing campaign the yield on step 1 was very consistent from batch to batch. Assay analysis of the step 1 extract was discontinued after the first several batches, and 0.95 equiv (relative to L-ribose) of **3** was charged in each batch throughout the campaign with good results.

At this point in development, a basic process for the step had been established as follows: to a mixture of step 1 extract and 1.05 equiv of **3** was added 0.56 equiv of acetic anhydride. The mixture was concentrated to remove all the solvents. To this mixture was added 1.0−1.85 mol % of triflic acid, and the batch was stirred at 110−130 °C under 20−65 mbar until the reaction was complete. The mixture was then cooled to around 70 °C. Methanol or ethanol was added, and the mixture was stirred at the temperature until a homogeneous solution was formed. The batch was then cooled to around 45 °C and seeded. The mixture was further cooled to below 10 °C. The solid was filtered, washed, and dried to give the step product, **1**.

The process was further optimized. The effects of two major parameters, reaction temperature and the amount of triflic acid, were studied. The reaction time was affected by both temperature and the amount of triflic acid. As illustrated in Figure 6, the reaction rate increased as the reaction temperature and/or the amount of triflic acid increased. For instance, the slowest batch took 15 h (at 105 °C and using 0.7 mol % of triflic acid), while the fastest reaction took only 45 min (at 130 °C and 3.0 mol % of triflic acid).

The effects of the temperature and triflic acid on the yield are illustrated in Figure 7.[11] Higher isolated yield could be obtained when the reaction was carried out at high temperature and at low dose of triflic acid. If only the isolated yield is considered when choosing the process parameters, the reaction should be carried out at around 130 °C using 0.5 mol % triflic acid. However, it was observed during the studies that the products were significantly darker in color when the reactions were carried out at temperatures higher than 125 °C or when a high dose of triflic acid was charged. Based on Statistical Experimental Design, optimum results for the process (such as reaction rate, product purity and color, overall yield, etc.) were achieved under the following conditions: temperature, 110−120 °C; triflic acid, 0.7−1.5 mol % (identified by the box in Figure 7). Above and to the right of this area, the purity of the product deteriorated and the product became darker in color. Below and to the left of this area, the reaction rate became slow.

In the original Ribavirin process, methanol was used in the crystallization of the step 2 product. Some concerns were raised during the evaluation of the process in our lab. For instance, at the target concentration (440 g crude step product per liter methanol) the slurry was very thick. This might cause operational difficulties during the transfer of the batch from reactor to centrifuge due to pipeline blockage or wall-cake formation inside the reactor. Increasing the methanol charge would result in yield loss because of the high solubility of the product in methanol (45 g/L at 20 °C). Also, seeding was required to induce the initial precipitation. Thus, ethanol and 2-propanol (IPA) were evaluated as candidates to replace methanol in the process. The solubility of **1** in IPA and ethanol is significantly lower than in methanol (5.5 g/L of IPA and 15 g/L of EtOH). The replacement of methanol with IPA or ethanol would allow higher solvent charge, and the slurry would not be as thick. Yield improve-

(11) The graph was generated through a DOE method. Program used: Version 3.1 of JMP. Method: Response Surface/central composite-orthogonal. Factors: reaction temperature (105−135 °C) and wt % of triflic acid (0.7−3.0%).

Table 5. **Crystallization of 1**

entry	solvent	volume, mL/g step 1 product	yield %[a]	wt% assay
1	MeOH	2.2	70.9	96.7
2	IPA	2.9	82.2	84.6
3	IPA	3.5	82.5	91.3
4	IPA	3.9	79.9	92.3
5	IPA	3.9	79.9	92.1
6	IPA	4.4	79.1	93.2
7	EtOH	2.9	77.1	94.7

[a] Overall yield from L-ribose.

ment was also possible. Thus, the three alcohols were compared side by side for the crystallization.

Higher yield was achieved using IPA at a concentration of up to 4.4 mL/g crude product (compared with 2.2 mL/g crude product in methanol). However, the purity of the crystallized product was lower, and the product was much darker in color. More importantly, it appeared that the transferability of the crystallization slurry in IPA was not better than that in MeOH, even though the concentration of the batch in IPA was only half of that in methanol. Although more IPA could be used to dilute the slurry without significantly sacrificing the yield, the approach would increase the operational volume at this point which was already the bottleneck volume of the whole process. The results in ethanol were between those in methanol and IPA. At the concentration of 2.9 mL/g crude product, ethanol offered comparable overall yield to that from IPA. The quality (color and wt % assay) of the products from ethanol were better than the ones from IPA but was not as good as the ones from methanol. Some examples are listed in Table 5. At the final concentration, the slurry in ethanol showed better transferability. As a compromise of product quality and yield, ethanol was chosen for the process. The experiments seemed to indicate that, in the case of ethanol, better yield could be achieved at lower hold temperature, so −5 °C was adopted for the process. Because the solubility of **1** in ethanol was low, the yield loss would be small with increased solvent charge. Therefore, when maximum operating volume is not an issue in the process, more ethanol should be used to improve the transferability of the batch.

Four lab demonstration batches were carried out on a scale of 100 g L-ribose. The overall yield was 72−74%. The major impurity was the unreacted **3** at 1.2−1.8%. The process was piloted on 100 kg of L-ribose scale. Six batches were carried out, with the average yield being 71.0% and average level of **3** being 1.87%. In a subsequent manufacturing campaign, 16 batches were conducted on the same scale, and the average yield was 74%. No major operational issues were encountered in both campaigns.

Experimental Section

Procedure for the Overall Process.[12] A dry, clean, 1 L, four-neck round-bottom flask was charged with 100 g of L-ribose and 500 mL of methanol. The mixture was stirred at 20 °C while 9.6 g of 95% sulfuric acid were slowly added. After the addition the mixture was stirred at 20 °C for 3 h. To this mixture was slowly added lithium carbonate (11.7 g), and the mixture was stirred for 30 min. Methanol (320 g) was distilled out under reduced pressure (bath temperature: 45 °C, vacuum: 100 mbar). To the mixture was added acetic acid (360 g), and the distillation was continued until 340 g of distillate were collected (vacuum: 50−100 mbar, bath temperature: 63 °C, pot temperature should be controlled not to exceed 52 °C). The mixture was sampled and analyzed for methanol and water content. The total methanol/water content should be below 3.5% (w/w). The bath temperature was lowered to 50 °C, and 251.6 g of acetic anhydride were added. After the addition the mixture was held for 1 h and then heated to 100 °C and held for 4 h. The mixture was then cooled to 20 ± 5 °C (pot temperature), and 52.6 g of 95% sulfuric acid were slowly added. The addition rate should be controlled so as to ensure that the pot temperature is 20 ± 5 °C. After the completion of the addition the mixture was stirred for 30 min at 20 ± 5 °C. Then, 95.2 g of acetic anhydride were slowly added in 2 h while maintaining the pot temperature at 20 ± 5 °C. After the addition the mixture was stirred at 20 ± 5 °C (pot temperature) for 30 min.

To a 1 L beaker was added 52.1 g of lithium carbonate and 100 mL of acetic acid. This mixture was stirred and cooled with an ice−water bath. The above reaction mixture was drained from the reactor into the beaker with good stirring. The whole mixture was then transferred back to the reactor and was concentrated under reduced pressure until over 419 mL of distillate were collected (vacuum: 60 mbar, bath temperature: 60 °C, final pot temperature: 57 °C). The mixture was cooled to 25 ± 5 °C, and to it was added dichloromethane (150 mL) and water (400 mL). The mixture was stirred at moderate speed for 30 min. The stirring was stopped, and the mixture was held for 15 min. The organic phase was separated, and to the aqueous layer was added another 150 mL aliquot of dichloromethane. The mixture was stirred at moderate speed for 15 min and then held for 15 min. The organic phase was separated. Both organic layers were combined and washed with 160 mL of 4% sulfuric acid. The pH of the aqueous phase should be below 2 at this point. The organic phase was separated as a clear light-yellow solution.

A 2 L flask was charged with 81 g of **3**, the above step 1 solution, and 37 g of acetic anhydride at ambient temperature. The mixture was distilled at atmospheric pressure (bath temperature, 90 °C). When the pot temperature reached 85 °C and the distillation became very slow, vacuum was applied (up to 30 mbar) and the distillation was continued for 40 min at 90 °C (bath temperature) and then for another 40 min at 120 °C (bath temperature, the pot temperature reached 117 °C). The vacuum was released, and 843 mg of triflic acid was slowly added. After the addition the vacuum was restored and the mixture was stirred at 115 ± 5 °C (pot temperature) for 4 h. Upon the completion of the reaction the mixture was cooled to 70 °C and to it was added ethanol (750 mL). When a homogeneous solution was

(12) Dong, Z.; Zhang, P. U.S. Pat. Appl. Publ. U.S. 2004034213.

formed the mixture was cooled to 50 °C and held until heavy precipitation formed (seeding might be necessary). The mixture was then slowly cooled to −5 °C (bath temperature) in 2 h and held for at least 2 h. The solid was filtered, washed with 100 mL of cold ethanol to give 250.9 g of step 1 product as a wet cake. Wt% assay was 75.5%, and overall yield was 74%.

Preparation of Pure 2 and Pure 4. To a 1 L, dry, clean, round-bottom jacketed flask was added 50.0 g of L-ribose and 400 g of anhydrous methanol. To this mixture was added 95% sulfuric acid (4.60 g). After the addition the mixture was stirred at ambient temperature for 3 h. To the vessel was added lithium carbonate (5.85 g) in one portion, and the mixture stirred at ambient temperature for 30 min. The mixture was subject to vacuum distillation (bath temperature: 30 °C) until 320 g of methanol were collected. The distillation was stopped, and 103 g of acetic acid were added. The vacuum distillation was reassumed (at bath temperature 40 °C) until 89 g of distillate was collected. The distillation was again stopped, and 146 g of acetic acid were added. Vacuum distillation was reassumed at bath temperature 40 °C and then slowly increased to 50 °C to distill out about 140 g of liquid. To this mixture was added acetic anhydride (125.8 g). The mixture was heated to 100 ± 5 °C and held for 5 h. The mixture was then cooled to 20 °C, and to it was slowly added 26.3 g of 95% sulfuric acid over 30 min, while maintaining the pot temperature below 25 °C. After the addition the mixture was stirred for 30 min at 20 ± 5 °C. A 47.6 g amount of acetic anhydride was added slowly over 2 h at 20 ± 5 °C. After addition, the contents were stirred for 1 h. To this mixture was slowly added 26.05 g of lithium carbonate. After the addition the mixture was stirred for 30 min. The mixture was subject to vacuum distillation at a bath temperature of 50 °C until about 150 g of liquid were collected.

To a 3/5 portion of the above residual content was added water (60 g). The mixture was stirred for 30 min at 50 °C and then cooled to 20 °C over 1 h and held for at least 30 min. To the slurry was added slowly a mixture of 30 g of 2-propanol and 120 g of water over 1 h. The mixture was then further cooled to 0−5 °C and aged for at least 2 h. The solid was filtered, washed with 72 g of water, and dried under high vacuum at 40 °C for 24 h to afford 38.27 g (60.2% corrected yield from L-ribose) of pure **2** as a white solid.

The mother liquor obtained above was extracted with 2 × 100 mL of 3:7 (V/V) mixed solvents of EtOAc/TBME. The combined organic layers were concentrated to almost dryness. The residue was subjected to an azeotropic distillation with 20 mL of toluene to remove residual water. The resulting mixture (13 g) was a colorless oil that contained a 3:1 mixture of **4** and **2**. Part of the mixture (12 g) was subjected to flash column chromatography (140 g silica gel), eluting with mixed solvents of EtOAc/petroleum ether (9:31 V/V), to give 4.8 g of **4** (97.1% area purity by GC analysis) as a colorless oil.

Reaction of 3 with 4. A 250 mL flask was charged with **3** (1.92 g) and a solution of **4** (4.8 g) in methyl acetate (50 mL). The mixture was concentrated at atmospheric pressure to almost dryness (bath temperature: 90 °C).To this mixture was added a solution of 22.7 mg of triflic acid in 1 mL of methyl acetate. The mixture was stirred at 115 ± 5 °C (pot temperature) under vacuum (30 mbar) for 4 h. Upon the completion of the reaction the mixture was cooled to 70 °C, and to it was added ethanol (23 mL). When a homogeneous solution was formed the mixture was cooled to 50 °C and held until a heavy precipitate formed. The mixture was then slowly cooled to −5 °C (bath temperature) in 2 h and held for 13 h. The solid was filtered, washed with 20 mL of cold ethanol, and dried under vacuum at 50 °C for 17 h to give 4.1 g (70% yield) of **1** as an off-white solid.

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