JOC Article

Practical Synthesis of a Potent Hepatitis C Virus RNA Replication Inhibitor

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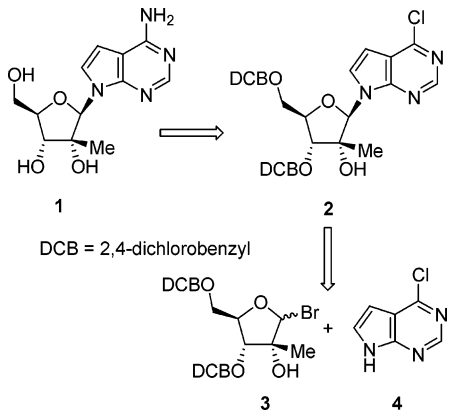
A practical, efficient synthesis of **1**, a hepatitis C virus RNA replication inhibitor, is described. Starting with the inexpensive diacetone glucose, the 12-step synthesis features a novel stereoselective rearrangement to prepare the key crystalline furanose diol intermediate. This is followed by a highly selective glycosidation to couple the C-2 branched furanose epoxide with deazapurine.

Introduction

Hepatitis C virus (HCV) currently infects 3.9 million Americans leading to 8 000−10 000 deaths annually as a result of chronic liver disease. Furthermore, infection by HCV is currently the leading indication for liver transplants. Co-infection of HIV patients with HCV averages about 70% with the result that HCV-related liver disease is a major reason for hospitalization and death among HIV-infected persons. The current HCV infection treatment protocols include interferon-α either alone or in combination with Ribavirin. While these regimens have been somewhat effective, not all patients respond to these drug therapies[1] and greater than 10% experience mild to moderate side effects.[2] Given the current state of the art and the fact that there is no vaccine available for the prevention of HCV,[3] it is clear that new protocols for the treatment of HCV infection are needed.

HCV is an enveloped virus with a single-stranded, positive-sense RNA genome. The HCV RNA encodes a handful of life-cycle essential enzymes including an RNA polymerase. As part of the process to replicate new viral particles, RNA polymerase acts to assemble copied portions of the viral RNA sequence into a single strand prior to encapsulation. In light of the key role HCV polymerase plays in the HCV life cycle, inhibition of this enzyme is a potential therapeutic strategy for treating HCV-infected patients. During a collaborative effort between Merck and ISIS Pharmaceuticals, the unnatural nucleoside **1** was identified as a potent inhibitor of HCV replication.[4] To provide the quantities of nucleoside **1** necessary for further biological testing, an efficient and practical synthesis of **1** was required.

SCHEME 1



Our initial foray into this chemistry began with the synthesis of **1** via the route disclosed by the Merck-ISIS collaboration.[5] The synthesis follows the logical, convergent retrosynthesis outlined in Scheme 1. Disconnection of **2** at the anomeric center reveals the commercially available, but prohibitively expensive, 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine **4**[6] and 3,5-bis-*O*-(2,4-dichlorobenzyl)-2-*C*-methyl-α-D-ribosylbromide **3**. While this route proved adequate for the small-scale preparation of **1**, providing material for further studies via this route would present some difficulties. As such, a more practical and efficient synthesis of **1** from readily available starting materials was needed. In particular, we sought a synthesis that would provide key intermediates as crystalline solids to facilitate purification. We required an efficient and stereoselective glycosidation reaction. The ultimate route we developed achieved each of these goals.

(1) Rosen, H. R.; Gretch, D. R. *Mol. Med. Today* **1999**, *5*, 393.
(2) Centers for Disease Control and Preventions. Recommendations for prevention and control of hepatitis C virus (HCV) and HCV-related chronic disease. In *Morbidity Mortality Weekly Rep.* **1998**, *47*, 1.
(3) Lemon, S. M.; Thomas, D. L. *New Engl. J. Med*. **1997**, *336*, 196.
(4) Carroll, S. S.; Lafemina, R. L.; Hall, D. L.; Himmelberger, A. L.; Kuo, L. C.; Maccoss, M.; Olsen, D. B.; Rutkowski, C. A.; Tomassini, J. E.; An, H.; Bhat, B.; Bhat, N.; Cook, P. D.; Eldrup, A. B.; Guinosso, C. J.; Prhavc, M.; Prakash, T. P. Patent WO 02/057425 A2, 2002.
(5) Carroll, S. S.; Maccoss, M.; Olsen, D. B.; Bhat, B.; Bhat, N.; Cook, P. D.; Eldrup, A. B.; Prhavc, M.; Song, Q. Patent WO 02/057287 A2, 2002.
(6) Chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine, also known as 6-chloro-7-deazapurine is available from Chem Genes, 299 Homer Av., Ashland, MA 01721. For a synthesis of 6-chloro-7-deazapurine see: Davoll, J. *J. Chem. Soc.* **1960**, 131.

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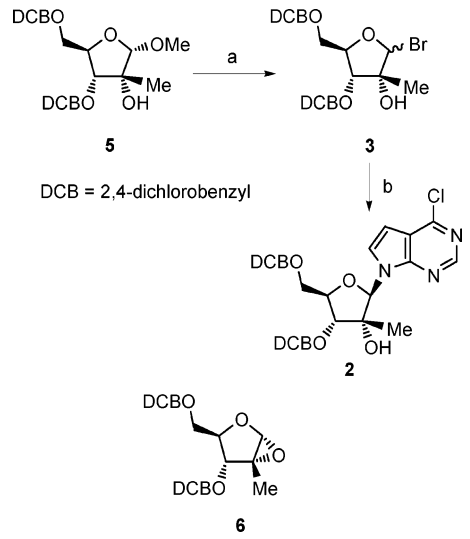
We wish to report a novel synthesis of **1** making use of a highly selective ester migration and a highly stereoselective glycosidation via a nucleophilic epoxide ring opening. Furthermore, the synthesis of **1** is made convergent by use of a protected form of 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine in the glycosidation coupling. Finally, a much-improved synthesis of the required 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine was developed.

Results and Discussion

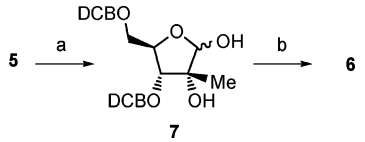
Glycosidation via Epoxide Intermediates. We initiated our studies by repeating the previously described synthesis of **1**, paying particular attention to the key glycosidation reaction. Treating intermediate **5** with 38% HBr−AcOH in dichloromethane resulted in formation of the corresponding ribosyl bromide **3** as a 5:1 mixture of α/*β*-anomers, as determined by ^{1}H NMR. Following concentration of the reaction solution to remove excess HBr−AcOH below 20 °C, the mixture of ribosyl bromides was treated with 1.5 equiv of the sodium salt of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (generated in situ). Despite the modest 43% yield of **2** observed in this reaction, we were pleased to confirm that none of the α-anomer of **2** was observed. If the glycosidation were proceeding via simple S_N2 displacement of bromide, we would have seen a 5:1 ratio of *β*/α anomers. In situ ^{1}H NMR experiments revealed that the reaction took place not through bromide displacement but through an intermediate epoxide (**6**).[7] It is known that nucleosides can be prepared by treatment of 1,2-anhydro sugars with heterocyclic bases.[7b,8] S_N2 opening of the α-epoxide of a 1,2-anhydro sugar results in highly selective formation of the *β*-nucleoside. Application of this method, however, in the preparation of nucleosides has been limited.[8] By carefully monitoring the reaction by HPLC and ^{1}H NMR, it was found that the moderate glycosidation yield is mainly due to significant cleavage of the 2,4-dichlorobenzyl groups in the presence of HBr−HOAc. On the basis of this understanding of the glycosidation reaction, we developed a route to the α-epoxide, eliminating the need for HBr, which was incompatible with the benzyl ether protecting groups. Hydrolysis of the 1-α-*O*-methyl ribose **5** gave diol **7**, which upon treatment with methanesulfonic anhydride, triethylamine, and DMAP gave epoxide **6**.[9] Treatment of the resulting epoxide solution with 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine and sodium hexamethyldisilazane gave a 65% yield of the desired **2**.

SCHEME 2[a]



[a] Reagents: (a) HBr/AcOH; (b) 6-chloro-7-deazapurine, NaH.

SCHEME 3[a]



[a] Reagents: (a) TFA/H_2O; (b) Ms_2O, Et_3N, cat. DMAP.

Despite the improved glycosidation yield, we still desired crystalline intermediates for ease of purification in large-scale preparation.

Vorbrüggen Route. Contemporaneous with our study of the route based on the previously described synthesis, we were also pursuing an alternate route to **1** based on the widely used Vorbrüggen glycosidation conditions.[10] From our review of the literature concerning the synthesis of 2-*C*-methyl ribonucleosides, we were cognizant of recent work by both the Wolfe[11] and Piccirilli[12] groups. Each group had reported the synthesis, under nearly identical conditions, of a series of closely related analogues of **1** in which only the nucleoside base differed.[11,12] The stereochemical control at the anomeric center observed in the Wolfe and Piccirilli syntheses was proposed to arise from neighboring group participation of the α-2-*O*-benzoate under Vorbrüggen reaction conditions. The Vorbrüggen reaction calls for the treatment of a peracylated carbohydrate with a strong Lewis acid such as TMSOTf, resulting in the formation of an oxonium ion intermediate (**8** → **9**). The resulting oxonium ion **9** then reacts with a silylated heterocyclic base to give *β*-nucleoside **10**. The selectivity in this reaction results from coordination of the neighboring 2-*O*-benzoate on the

(7) Formation of an epoxide in the reaction of C-2 hydroxyl-furanosyl halides with base was proposed previously. For examples, see: (a) Reist, E. J.; Hart, P. A.; Goodman, L.; Baker, B. R. *J. Am. Chem. Soc.* **1959**, *81*, 5176. (b) Jarman, M.; Ross, W. C. J. *J. Chem. Soc.* (*C*) **1969**, 199.

(8) For examples, see: (a) Ning, J.; Xing, Y.; Kong, F. *Carbohydr. Res.* **2003**, *338*, 55. (b) Jung, M. E.; Toyota, A. *Terahedron Lett.* **2000**, *41*, 3577−3581. (c) Ning, J.; Kong, F. *J. Carbohydr. Chem.* **1997**, *16*, 311. (d) Ning, J.; Kong, F. *Carbohydr. Res.* **1997**, *300*, 355. (e) McDonald, F. E.; Gleason, M. M. *J. Am. Chem. Soc.* **1996**, *118*, 6648. (f) Baumgartner, H.; Marschner, C.; Pucher, R.; Griengl, H. *Tetrahedron Lett.* **1991**, *32*, 611. (g) Chow, K.; Danishefsky, S. *J. Org. Chem.* **1990**, *55*, 4211. (h) Biggadike, K.; Borthwick, A. D.; Exall, A. M.; Kirk, B. E.; Roberts, S. M.; Youds, P. *J. Chem. Soc., Chem. Commun.* **1987**, 1083. (i) Konno, K.; Hayano, K.; Shirahama, H.; Saito, H.; Matsumoto, T. *Tetrahedron* **1982**, *38* 3281. (j) Holý, A.; Šorm, F. *Collect. Czech. Chem. Commun.* **1969**, *34*, 3383.

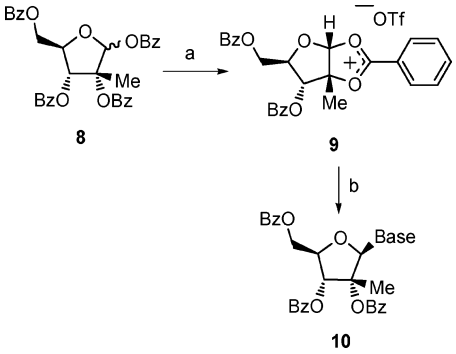
(9) The initially formed mixture of anomeric mesylates gave only the α-epoxide, likely via a solvolysis mechanism. When the reaction is run at room temperature epoxide formation is rapid, but at lower temperatures both α- and *β*-mesylate anomers can be observed as precursors to the epoxide.

(10) For an extensive review of this reaction see: Vorbrüggen, H.; Ruh-Pohlenx, C. *Org. React.* **2000**, *55*, 1.

(11) Harry-O'kuru, R. E.; Smith, J. M.; Wolfe, M. S. *J. Org. Chem.* **1997**, *62*, 1754.

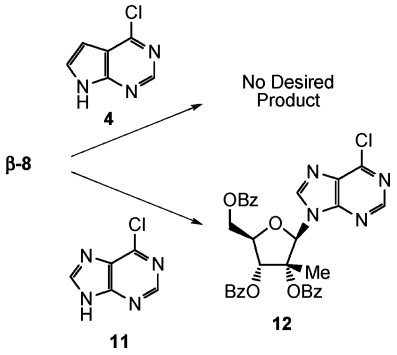
(12) Tang, X.-Q.; Liao, X.; Piccirilli, J. A. *J. Org. Chem.* **1999**, *64*, 747.

SCHEME 4[a]



[a] Reagents: (a) TMSOTf; (b) persilylated base.

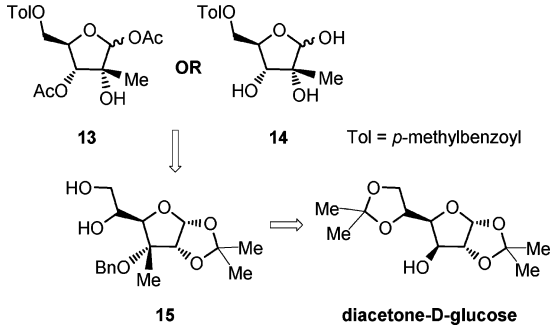
SCHEME 5



α-face, effectively directing attack of the silylated nucleophile to the β-face. Given the close analogy between the Wolfe and Piccirilli targets and our own, we naturally chose to investigate the synthesis of **1** using Vorbrüggen conditions. We began with the reported three-step synthesis of **8** from commercially available 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose, repeated with only minor modification.[13] Attempts to use β-**8** as the glycosyl donor in reaction with 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine under a variety of Vorbrüggen conditions failed to give the desired product.[14] To confirm that the failure was specific to the 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine, the reaction was performed with 6-chloropurine **11** and gave a near quantitative yield of the expected β-nucleoside **12**.[15] While it appeared that we were both silylating the 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (as evidenced by in situ ^{1}H NMR) and forming the oxonium ion intermediate, no conditions were found to effect the desired glycosidation.

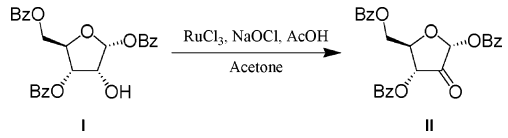
Though the Vorbrüggen chemistry proved incompatible with the pyrrolo[2,3-*d*]pyrimidine base, we were pleased to find that the benzoate-protected carbohydrates such as β-**8** were crystalline. To combine the favorable features in both approaches, we subsequently sought to design a route that would rely on ester protection to give crystalline intermediates leading to a β-selective glycosidation via the epoxide.

SCHEME 6



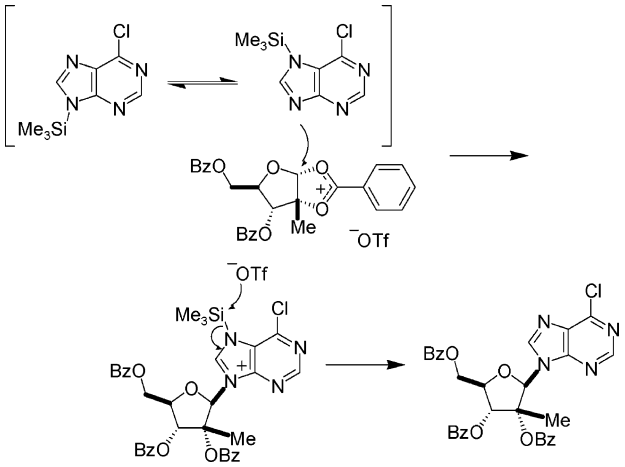
The Diacetone-D-glucose Route to the Epoxide. Seeking a route to a diol analogous to **7** but bearing ester-protected 3,5-hydroxyls, we were aware of reports of the 2-*C*-methyl-D-ribose structure mapped back to commercially available diacetone-D-glucose (Scheme 6).[16−18] We were further encouraged by the report of a few crystalline intermediates en route from diacetone-D-glucose to **13**. As per the literature reports, we found the 10-step synthesis gave **13** in about 40% overall yield from

(13) See ref 11. The oxidation of 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose (**I** → **II**) was modified from the original Dess−Martin periodate oxidation to a $RuCl_3$ catalyzed bleach oxidation.



(14) The β-anomer of **8** could be isolated from the anomeric mixture by selective crystallization. The formation of the oxonium ion intermediate **9** from the β-anomer upon treatment with TMSOTf was found to be significantly more rapid than that with the α-anomer.

(15) Literature concerning the mechanism of Vorbrüggen glycosidation suggests a reason for the nonreactive nature of 6-chloro-7-deazapurine.



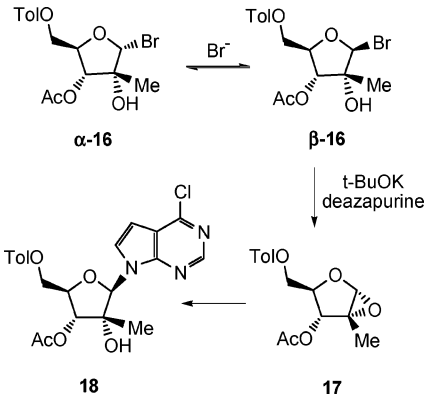
Vorbrüggen has proposed that the attack of the silylated base on the intermediate oxonium takes place through the nucleophile β to the silane rather than in an ipso sense. In the case of the deazapurine it is unlikely that silyl migration to the 7-*C* occurs, thus only reaction through one of the remotely conjugated, silylated pyrimidine nitrogen atoms may be invoked. Then again, there are a number of literature examples of Vorbrüggen glycosidation successfully employed for nucleophiles that do not fit well with this mechanistic rationalization. For an extensive discussion of the mechanism of reaction of silylated purines with similar oxonium ions, see ref 10. Also insightful are the following: Dempcy, R. O.; Skibo, E. B. *J. Org. Chem.* **1991**, *56*, 776. Křen, V.; Pískala, A.; Sedmera, P.; Havlíček, V.; Přikrylová, V.; Witvrouw, M.; De Clercq, E. *Nucleosides Nucleotides*, **1997**, *16*, 97. Other examples may be found in the tables given in ref 10.

(16) Brimacombe, J. S.; Rollins, A. J.; Thompson, S. W. *Carbohydr. Res.* **1973**, *31*, 108.

(17) Funabashi, M.; Yamazaki, S.; Yoshimura, J. *Carbohydr. Res.* **1975**, *44*, 275.

(18) Beigelman, L. N.; Ermolinsky, B. S.; Gurskaya, G. V.; Tsapkina, E. N.; Karpeisky, M. Y.; Mikhailov, S. N. *Carbohydr. Res.* **1987**, *166*, 219.

SCHEME 7

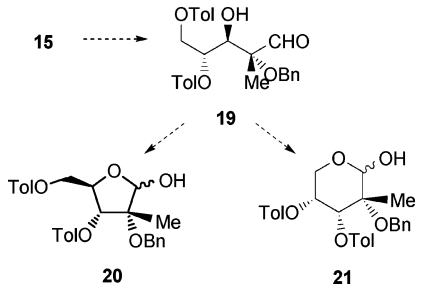


diacetone-D-glucose. Unfortunately, **13** was isolated as an oil. With only minor modification of the published route, however, we could access the *crystalline* intermediate **14**.

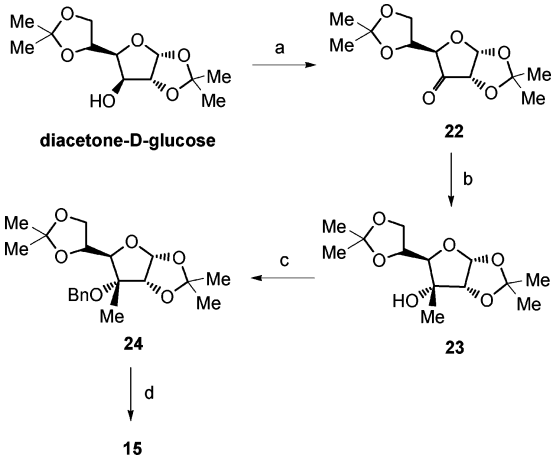
We found that both **13** and **14** could serve as glycosidation precursors as treatment of either with HBr−AcOH gave the same ribosyl bromides **16**.[19] We noted that at the end of reaction the bromides were present as an 11:1 mixture of anomers favoring the α-**16**. Upon concentration with toluene to remove excess HBr−AcOH, however, the ratio degraded to 4:1 α/*β*. Glycosidation of these bromides with potassium *tert*-butoxide and 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine gave only the desired *β*-anomer **18**. ^{1}H NMR showed once again that the glycosidation conditions were converting both bromides to an epoxide intermediate, in this case **17**, which then reacted to give the desired *β*-glycosidation product **18** in about 62% overall yield from triol **14**. The use of HBr−AcOH to access epoxide **17** was operationally less than optimal, requiring azeotropic removal of excess reagent. Additionally, the 3-acetate appeared to be somewhat labile under the glycosidation conditions. Loss of the acetate led to formation of what appear to be dimeric and oligomeric products. From what we had learned to this point, we realized that the ideal substrate for the glycosidation would be a 1,2-diol allowing mild and efficient epoxide formation via mesylation. Additionally, the ideal substrate would possess robust protecting groups on the 3 and 5 hydroxyls. It occurred to us that it might be possible to access such a substrate using a sequence analogous to the one used to prepare **14** if **15** were esterified at the start to give the diester rather than the monoester. Esterification of both hydroxyls of **15** followed by acetonide hydrolysis, oxidative cleavage, and selective formyl hydrolysis would give **19**. Success would then require defining conditions promoting acyl migration and affording furanose **20** rather than pyranose **21**.[20] Subsequent hydrogenolysis of the benzyl ether would afford a 1,2-diol substrate for use in the glycosidation. To determine the potential for success in this strategy, aldehyde **27** was prepared from the diester **25** by a procedure similar to the one developed for preparation of **14**.

(19) Treatment of **14** with HBr−AcOH resulted in complete bromination at the anomeric position but only ~85% of the bromide was acylated at the 3-OH despite the use of excess HBr−AcOH. While no further work was done to elucidate the mechanism of 3-OH acylation, our observations suggest that intramolecular transfer of a 1-*O*-acyl to the 3-OH is involved.

SCHEME 8



SCHEME 9[a]

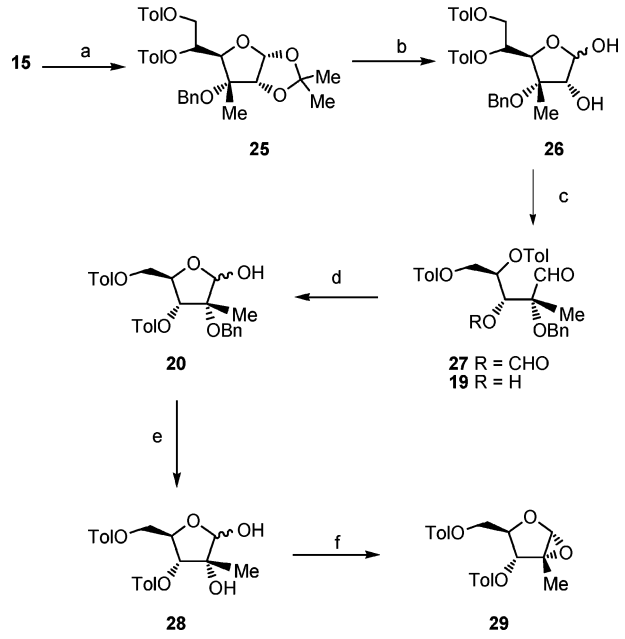


[a] Reagents: (a) 1 mol % of TEMPO, NaOCl, NaBr, NaOAc; (b) MeMgCl; (c) KOH, Triton X-405, BnCl; (d) 5% aq H_2SO_4.

Treatment of **27** with Et_3N in MeOH at 60 °C gave the desired furanose in 90% selectivity (**20**:**21** = 9:1). This was quite an encouraging result for the first trial. We were pleased to find that the diol **28**, obtained upon hydrogenolysis of **20**, is a crystalline solid. Subjecting **28** to reaction with methanesulfonic anhydride and triethylamine gave the desired epoxide **29**, which reacted with the 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine to give the *β*-glycosidation product. In light of these results, we set about to optimize the synthesis of **1** via the crystalline diol **28**. A number of steps in the literature synthesis from which we were working proved inefficient or impractical for the preparation of **1**. Specifically, the oxidation of diacetone-D-glucose to give ketone **22** called for the use of excess pyridinium dichromate (PDC) and 300 wt % powdered 4 Å sieves.[21] The use of molecular sieves to absorb water was required; otherwise, a mixture of

(20) Acyl migration is well-known in carbohydrate chemistry where the migration takes place on a rigid framework and has been used to prepare compounds that would be difficult to attain by direct esterification. (a) Haines, A. H. Relative Reactivities of Hydroxyl Groups in Carbohydrates. In *Advances in Carbohydrate Chemistry and Biochemistry*; Tipson, R. S., Horton, D., Eds.; Academic Press: New York, 1976; Vol. 33, pp 11−109. (b) Horrobin, T.; Tran, C. H.; Crout, D. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1069. (c) Goueth, P. Y.; Gogalis, P.; Bikenga, R.; Gode, P.; Postel, D.; Ronco, G.; Villa, P. *J. Carbohydr. Chem.* **1994**, *13*, 249. (d) Chaplin, D.; Crout, D. H.; Bornemann, S.; Hutchinson, D. W.; Khan, R. *J. Chem. Soc., Perkin Trans. 1* **1992**, 235. There are fewer examples where acyl migration has been employed in an acyclic system. (e) Suh, Y.-G.; Jung, J.-K.; Seo, S.-Y.; Min, K.-H.; Shin, D.-Y.; Lee. Y.-S.; Kim, S.-H.; Park, H.-J. *J. Org. Chem.* **2002**, *67*, 4127. (f) Vares, L.; Rein, T. *Org. Lett.* **2000**, *2*, 2611. (g) VanMiddlesworth, F.; Lopez, M.; Zweerink, M.; Edison, A. M.; Wilson, K. *J. Org. Chem.* **1992**, *57*, 4753. (h) Maycock, C. D.; Barros, M. T.; Santos, A. G.; Godinho, L. S. *Tetrahedron Lett.* **1992**, *33*, 4633.

SCHEME 10[a]



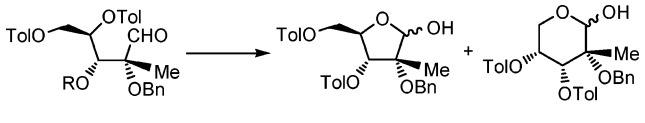
[a] Reagents: (a) toluoyl chloride, pyridine; (b) HBF_4; (c) H_5IO_6; (d) $^iPr_2NEt$, MeOH, ^{i}PrOAc; (e) H_2, Pd/C; (f) MsCl, Et_3N.

ketone and hydrate was isolated. These conditions are environmentally undesirable due to the large volume of chromium-contaminated waste produced. Furthermore, to isolate the ketone free from chromium required a filtration through either silica gel or Florosil, which also created chromium-laden waste. We found the alcohol oxidation could be accomplished using catalytic TEMPO with aqueous bleach as oxidant. This protocol gave a mixture of ketone and hydrate. Dehydration of a toluene solution by azeotropic distillation gave the ketone **22** as a solution in toluene. The resulting toluene solution was treated with methylmagnesium chloride at 0 °C to give **23** as a crystalline solid from toluene−octane in 85% overall yield from diacetone-D-glucose. Use of the dehydrated ketone is critical as the hydrated form does not react with methylmagnesium chloride to give the desired product but is returned as the hydrate upon quenching.

While published methods for benzylation of the 3-hydroxyl to give **24** call for the use of NaH in DMSO, we wished to avoid the use of NaH due to safety concerns. We found this benzylation could be accomplished by heating **23** in toluene with solid KOH, benzyl chloride, and Triton X-405 as the solid−liquid phase transfer catalyst. The crude benzyl ether product was subsequently treated with dilute acid to effect selective cleavage of the 5,6-acetonide. Under these optimized conditions, diol **15** is isolated by crystallization from toluene−heptane in a 84% yield from **23**. Reaction of **15** with toluoyl chloride in acetonitrile using pyridine as base afforded the diester **25**. After screening a variety of acids for hydrolysis of the acetonide, we found that aqueous

(21) A number of oxidations have been applied to this problem including some catalytic methods (see: Weber, J. F.; Talhouk, J. W.; Nachman, R. J.; You, T.-P.; Halaska, R. C.; Williams, T. M.; Mosher, H. S. *J. Org. Chem.* **1986**, *51*, 2702); most reports, however, rely on the high-yielding chromium-based oxidations such as: Shing, T. K. M.; Wong, C.-H.; Yip, T. *Tetrahedron*: *Asymmetry* **1996**, *7*, 1323.

TABLE 1. Conditions for Formation of 20



entry	base	solvent	conditions	conv, %	area % **21**[a]
1	Et_3N	MeOH/water	rt, 48 h	99	8
2	Et_3N	MeOH/water	60 °C, 5 h	97	11
3	Et_3N	MeOH	60 °C, 10 h	98	10
4	Et_3N	THF/water	60 °C, 48 h	54	12
5	Et_3N	THF	60 °C, 48 h	4	37
6	$^iPr_2NH$	MeOH	0 °C, 24 h	99	4
7	$^iPr_2NH$	MeOH/^{i}PrOAc	0 °C, 48 h	98	4
8	$^iPr_2NH$	MeOH	rt, 27 h	99	12
9	DMAP	MeOH	rt, 48 h	80	7
10	Imidazole	MeOH	45 °C, 72 h	45	4
11	$NaHCO_3$	MeOH	45 °C, 48 h	97	12

[a] HPLC area % **21** of total products (**20** + **21**).

HBF_4 gave **26** at a reasonable reaction rate, but the hydrolysis stalled at 90% conversion. By removing acetone liberated during hydrolysis by vacuum distillation, we were able to achieve a satisfactory 96% conversion. Treatment of diol **26** with periodic acid resulted in a mixture of **27** and **19**. To optimize the formation of **20**, we needed to identify conditions that would minimize the formation of pyranose **21.** After screening solvent, temperature, and base, we discovered that using 2 equiv of diisopropylamine in methanol−isopropyl acetate led to **20** with >23:1 selectivity over **21**.[22] Hydrogenolysis of the 2-benzyl ether gave the desired diol **28** as a crystalline solid from isopropyl acetate−hexanes in 65% overall yield from **15**.

With **28** in hand we found that epoxide formation could be effected with methanesulfonyl chloride in place of the more expensive methanesulfonic anhydride. The epoxide **29** turned out to be remarkably stable to hydrolysis, tolerating aqueous workup to give a 93% yield from **28**.[23] With an efficient synthesis of **29** now fully developed we turned our attention to the critical glycosidation reaction.

Parallel with our carbohydrate studies we were working to identify a nucleoside base which was compatible with the nucleophilic epoxide ring opening. We began our glycosidation studies with the commercially available, but prohibitively expensive, 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine. The chloro substituent was to be converted to the required amino function by ammonolysis after coupling.[24] The ammonolysis was expected to require a high temperature in alcoholic ammonia with use of a pressure-

(22) See entries **6** and **7** from Table 1. We chose the mixed solvent system, MeOH-^{i}PrOAc, despite the longer reaction times so that the solution of **27** and **19** in ^{i}PrOAc from the extractions following oxidative cleavage could be used directly without isolation of the product mixture or complete removal of ^{i}PrOAc as would be required to conduct the reaction in methanol alone.

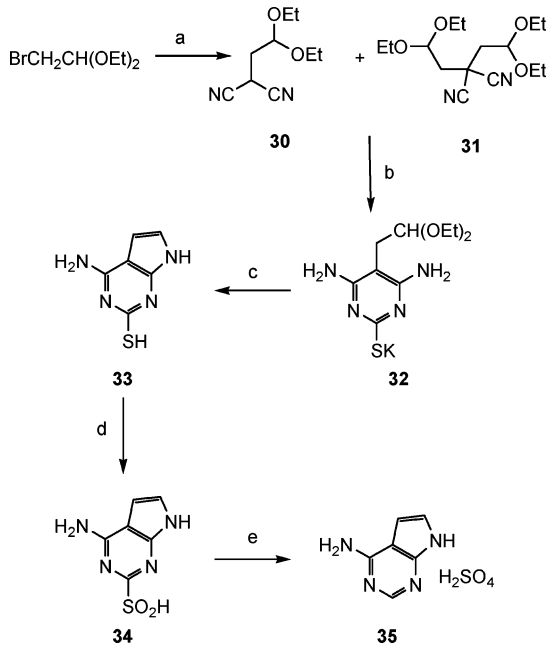
(23) Solutions of the epoxide proved unchanged after several weeks at room temperature. This should be contrasted with the stability of epoxides bearing 3,5 benzyl ethers (**6**), which readily hydrolyzed on exposure to water. We attribute this stability to the electron-withdrawing nature of the 3,5 esters which destabilize the formation of an oxonium ion. Such long-range effects have been noted and quantified in the carbohydrate literature. See: Glaudemans, C. P. J.; Fletcher, H. G., Jr. *J. Am. Chem. Soc.* **1965**, *87*, 4636. Green, L. G.; Ley, S. V. *Carbohydr. Chem. Biol.* **2000**, *1*, 427.

rated reactor. We hoped to avoid these forcing conditions and achieve a more convergent synthesis of **1** by going into the glycosidation with the amino group in place. To explore the glycosidation with various protected analogues of 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidines, we required an efficient synthesis of the parent nucleoside base.

Synthesis 4-Phthalimido-7*H*-pyrrolo[2,3-*d*]pyrimidine. A review of the literature concerning the 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine leads one back to two similar reports of its synthesis, one by Davoll[25] and the other the subject of a patent by the Wellcome Foundation Limited.[26]

The Davoll and Wellcome syntheses form the pyrimidine ring by condensation of a malonate derivative with thiourea and then remove the sulfur atom with Raney nickel. In both cases the best route to the 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine was through ammonolysis of the 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine, a process that called for extended high temperature and pressure exposure of the chloro compound to alcoholic ammonia. This reaction led to byproducts resulting from incorporation of the alcohol at the 4-position. The chloro compound was accessed through the 4-hydroxyl-7*H*-pyrrolo[2,3-*d*]pyrimidine by reaction of it with $POCl_3$, a process that generates a great deal of acid waste. For these reasons neither process seemed efficient. Herein we report an improved synthesis that, while based on the Wellcome and Davoll syntheses, includes important modifications leading to a practical, efficient synthesis of 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine. The reported procedures call for the reaction of bromoacetaldehyde diethyl acetal with malononitrile to be carried out in the presence of sodium ethoxide.[27] Optimization of base, solvents, and stoichiometry revealed that this alkylation could be performed in DMF with potassium carbonate, milder and more readily handled than sodium ethoxide. Under these conditions a 60% yield of **30** is realized while limiting over-alkylation to 10−15%. Following aqueous workup, the crude solution of **30** and **31** is treated with thiourea in the presence of potassium *tert*-butoxide to form the pyrimidine ring in 85%. The potassium salt **32** was conveniently isolated by crystallization from the reaction mixture, rejecting multiple side products and resulting in >95% pure product.[28] Use of the mixture of **30** and **31** in the reaction with thiourea obviated the need for a high-temperature vacuum distillation required to isolate **30** free from **31**. Isolation of the potassium salt instead of the neutral compound was found to be more convenient in that it eliminated the need for careful pH adjustments and gave higher purity product. Exposure of **32** to aqueous HCl results in near-quantitative conversion to **33**.

SCHEME 11[a]



[a] Reagents: (a) K_2CO_3, malononitrile; (b) thiourea, *t*-BuOK; (c) 5 N HCl; (d) H_2O_2; (e) EtOH, H_2SO_4.

A major limitation of both the Davoll synthesis and that reported by Wellcome is the need to use Raney nickel reduction for cleavage of the sulfur−carbon bond, a procedure that generates much heavy metal waste. Furthermore the reduction procedure requires a hot filtration at the end of the reduction due to the low solubility of the intermediate. We hoped to avoid these complications in the sulfur removal by making use of the observation that aromatic sulfinic acids readily lose sulfur dioxide upon heating with sulfuric acid.[29]

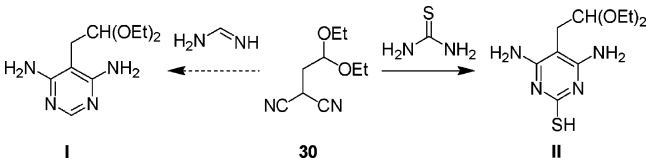
Sulfinic acid **34** was prepared by reaction of **33** in 1 N potassium hydroxide with 2 equiv of hydrogen peroxide. The oxidation was carefully controlled so that the hydrogen peroxide was charged at the optimal rate to minimize the formation of the sulfonic acid as a result of over-oxidation. We found that slow addition of hydrogen peroxide (3 h addition time) led to more over-oxidation than when shorter addition times were used.[30] On a large scale, the addition was done in portions as fast as possible while still controlling the temperature. The sulfinic acid **34** was then heated in 50 wt % sulfuric acid resulting in loss of SO_2 to give **35** as the hydrogen sulfate salt, which

(24) This conversion was reported previously in the synthesis of **1**.[5] Ammonolysis of a glycosylated 6-chloro-7-deazapurine was successfully performed to synthesize the natural product tubercidin: Anderson, J. D.; Botems, R. J.; Geary, S.; Cottam, H. B.; Larson, S. B.; Matsumoto, S. S.; Smee, D. F.; Robins, R. K. *Nucleosides Nucleotides* **1989**, *8*, 1201.

(25) Davoll, J. *J. Chem. Soc.* **1960**, 131.

(26) The Wellcome Foundation Limited, UK patent 812366, 1955.

(27) An obvious opportunity for improving pyrimidine synthesis would be to do the ring forming condensation not with thiourea but with formamidine. Despite attempts at this condensation under a variety of conditions we were never able to realize greater than 15% conversion to the desired **I**.

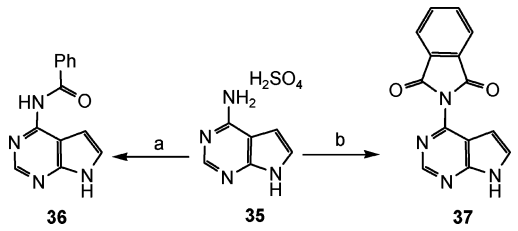


(28) HPLC analysis gave 95.6 area % at 210 nm.

(29) This desulfurization reaction has been used in the synthesis of other nitrogen heterocycles. Evans, R. M.; Jones, P. G.; Palmer, P. J.; Stephens, F. F. *J. Chem. Soc.* **1956**, 4106.

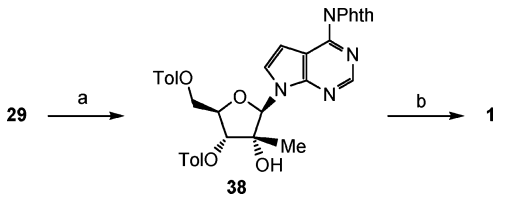
(30) Careful control of stoichiometry is important in the oxidation, and use of greater than 2 equiv of H_2O_2 results in over oxidation. It is not immediately clear why slower addition rates favor over oxidation. For an investigation into the mechanism of aryl thiol oxidation with H_2O_2 see: Evans, B. J.; Doi, J. T.; Musker, W. K. *J. Org. Chem.* **1990**, *55*, 2337.

SCHEME 12[a]



[a] Reagents: (a) PhCOCl; (b) phthalic anhydride, $^{i}Pr_2NEt$.

SCHEME 13[a]



[a] Reagents: (a) NaH, 6-phthalimido-7-deazapurine; (b) *n*-butylamine.

crystallized directly from the reaction mixture in 84% yield from **33**. The sulfate salt **35** served as a convenient intermediate for derivatization of the primary amino group. For example, reaction of **35** with benzoyl chloride in the presence of excess base gave the benzamide **36** in high yield. The desired 6-phthalimido-7-deazapurine **37** was prepared by treating **35** with phthalic anhydride and Hünig's base in dimethylacetamide at 80−85 °C to give **37** as a crystalline solid isolated in >85% yield.

Glycosidation and Deprotection. In seeking an appropriate protecting group for the amine functionality we first looked to amides, which might allow for deprotection simultaneous with deprotection of the esters at the 3 and 5 positions. While benzoyl protection is most obvious, its use gave rise to side products resulting from reaction through the pyrimidine portion of the base, a result we attributed to the acidic amide NH.[31] Employing phthalimide protection[32] eliminated the acidic amide NH and, consequently, the side products from pyrimidine reaction. With this new substrate we found that only a catalytic amount of NaH was required to achieve complete conversion of the epoxide.[33] While the phthalimide nucleoside base gave excellent yields for the C−N bond formation, the yield of **38** suffered due to the formation of products resulting from hydrolysis of the 3-ester, the imide, or both, despite our efforts to keep the reactions anhydrous.[34] The formation of this mixture turned out to be of little consequence, however, when it was found that heating the end of the reaction mixture with *n*-butylamine converted all the products to **1**.[35] The target nucleoside **1** was isolated following global deprotection by direct crystallized from solution on addition of antisolvent. In this manner diol **28** could be converted to nucleoside target **1** in 77% overall yield. The final product was conveniently isolated by crystallization from 1-propanol−water in >99% purity. The results presented here suggest that 1,2-anhydrofuranose carbohydrates may find fruitful employment in the synthesis of nucleosides containing heterocycles which are not amenable to the Vorbrüggen conditions.

Conclusion

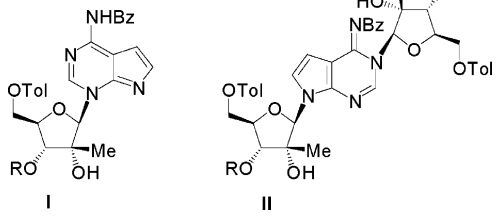
We have developed a practical, efficient, and convergent synthesis of nucleoside **1** via the crystalline 3,5-di-*O*-toluoyl-2-*C*-methyl-D-ribofuranose starting from the inexpensive and readily available diacetone-D-glucose. A catalytic TEMPO-bleach oxidation of diacetone-D-glucose to give 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose was developed as an environmentally sound alternative to the typical chromium oxidation. An ester migration was exploited to selectively give the 3,5-diester. The *β*-selective glycosidation of 3,5-di-*O*-toluoyl-2-*C*-methyl-D-ribofuranose has been developed via the nucleophilic opening of an 2-*β*-*C*-methyl-1,2-α-anhydroribose formed under mild, stereospecific conditions. Additionally, we have developed a practical synthesis of 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine. These methods have been applied to the multi-kilogram synthesis of the potential HCV polymerase inhibitor **1**, realized in 12 steps and 35% overall yield.

Experimental Section

General. All reactions were conducted under N_2 atmosphere with standard air-free manipulation techniques. Solvents and common reagents were purchased from a commercial source and used without further purification. Concentration in vacuo refers to removal of the solvent with a rotary evaporator at reduced pressure. Chemical shifts are reported in ppm downfield from tetramethylsilane (TMS). Data are reported as follows: (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; integration; coupling constant(s) in Hz; assignment). All new compounds are fully characterized below.

1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose (22). Water (10 kg) and ethyl acetate (30 L) were charged to a 100-L vessel followed by addition of diacetone-D-glucose (6.67 kg, 25.6 mol), sodium bromide (2.11 kg, 20 mol), sodium acetate (3.15 kg, 38.4 mol), and then TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) (40 g, 0.256 mol). Additional water (3.3 kg) and ethyl acetate (3 L) were charged and the resulting solution was cooled to 14 °C. Bleach (ca. 12.5% solution, 16.9 kg, 28.4 mol) was then added over 2 h maintaining the

(31) Specifically, we observed products such as **I** and **II**.



(32) Kume, A.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1982**, *23*, 4365−4368.

(33) As little as 20 mol % of NaH could be used to effect complete conversion of the epoxide. We routinely employed 50 mol % to ensure complete conversion.

(34) The mechanism of 3 deprotection or phthalimide opening was never clarified. These products, specifically the deprotection at 3, were observed regardless of rigorous exclusion of water from these reactions. The hydrolysis of 3 esters may be the result of a general base assistance by 2-hydroxyl (alkoxides) or acceleration due to DMAP-like attack by the pyrimidine heterocycle.

(35) Although phthalimide can be deprotected by hydrolysis in aq NaOH or methanolysis in NaOMe/MeOH, the following workup and isolation of **1** are not as straightforward as the use of *n*-$BuNH_2$.

temperature between 10 and 20 °C with constant cooling. After a 30-min age, ^{1}H NMR analysis indicated 1% residual starting material. The reaction was quenched with 2 M Na_2SO_3 aqueous solution (100 mL, 0.2 mol) after which a starch iodide test proved negative. The phases were separated and the aqueous phase was extracted with ethyl acetate (33 L). The combined organic extracts were washed with 12% aqueous NaCl solution (6.6 L). The major component in solution at this point is the ketone−hydrate. The resulting organic phase was concentrated under reduced pressure (pot temperature ca. 15 °C) to low volume. Toluene (35 L) was then added and the distillation was continued adjusting the vacuum until the pot temperature was 70 °C at which point azeotropic water removal was observed. When water evolution had ceased, the reaction was sampled and ^{1}H NMR analysis indicated complete break of hydrate (ca 0.5 equiv of residual ethyl acetate relative to product was observed). The solution volume was adjusted to 45 L by addition of toluene, and the resulting solution of **22** was used as is in the next step.[36]

^{1}H NMR Method for Oxidation/Hydrate Break. For determining the azeotropic dehydration end-point the NMR chemical shift of the anomeric ^{1}H proved diagnostic. For the hydrate break, a 200-μL sample of the toluene reaction mixture was added to 1 mL of $CDCl_3$ and analyzed immediately [species, δ anomeric ^{1}H]: diacetone-D-glucose, 5.95 ppm (J = 4.0 Hz); ketone (**22**), 6.14 ppm (J = 4.8 Hz); and hydrate, 5.85 ppm (J = 4.0 Hz).

1,2:5,6-Di-*O*-isopropylidene-3-*C*-methyl-α-D-allofuranose (23). Methylmagnesium chloride (10.2 L of a 3 M THF solution) was added over 80 min to the crude solution of ketone **22** in toluene (ca. 45 L) while the internal temperature was maintained in the range −10 to 0 °C. After a 30-min age, the reaction was quenched by addition to a solution of ammonium chloride (23.2 L of 4 M aqueous solution). The layers were separated and the aqueous phase was extracted with ethyl acetate (23 L). The combined organic phase (ca. 75 L) was washed with 12% NaCl aqueous solution (23 L) then filtered through a 10-μm in-line filter and concentrated under reduced pressure to a low volume (ca. 10 L). During the concentration, the product started to crystallize out. Octane (45 L) was added and the slurry was warmed to 78 °C dissolving the solids. The solution was allowed to cool and crystallization occurred at 50 °C. The mixture was cooled to −10 °C and filtered to afford 7.02 kg of tertiary alcohol **23** in 85% yield from diacetone-D-glucose.[21]

3-*O*-Benzyl-3-*C*-methyl-1,2-*O*-isopropylidene-α-D-allofuranose (15). A 72-L vessel fitted with a mechanical stirrer was charged with toluene (36 L), then solid alcohol **23** (4.32 kg, 15.75 mol), followed by benzyl chloride (2.92 kg, 23.1 mol) and Triton X-405 (70% solution in water, 0.45 kg). KOH powder (3.7 kg, 65.9 mol) was added in a single portion resulting in a mild exotherm (19 °C up to ∼30 °C). The reaction vessel was fitted with a Dean−Stark trap and heated to reflux for 20 h. Over the course of 20 h approximately 250−300 mL of water collected in the trap. Upon reaching >97% conversion (as determined by GC), the reaction was cooled to room temperature and washed with 10 L of water. The layers were separated and the organic was washed with 5 L of 1 M HCl and finally with 5 L of 12% aqueous NaCl solution. The resulting organic solution was then concentrated at reduced pressure to give 3-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-3-*C*-methyl-α-D-allofuranose (**24**) as a >70 wt % solution in toluene. This reaction was repeated at the same scale and combined to give an assay total of 10.81 kg of **24**, 29.66 mol, as a 70 wt % solution in toluene. The toluene solution was then diluted with acetonitrile (47 L) in a 100-L vessel with stirring. To this solution was added 14.5 L of a 5 vol % of sulfuric acid aqueous solution and the reaction temperature was maintained at ca. 19 °C. The reaction is initially cloudy but becomes homogeneous during the second hour and remains so. When HPLC indicated >98% conversion of **24** (approximately 4 h), 18 L of toluene was added causing phase separation. The aqueous phase was then cut and the organic layer washed with 20 L of ∼6% $NaHCO_3$ solution. Following the phase cut the organic phase was washed with 10 L of water. The organic layer was then concentrated at reduced pressure with the internal temperature of 50 °C to about 30 L. An additional 30 L of toluene was added and the solution was again concentrated to 30 L, allowing the temperature of the pot to rise to 80 °C. *n*-Heptane (43 L) was then slowly added while the internal temperature was maintained at about 80−75 °C. The solution was then cooled to 70 °C at which point product began crystallizing from solution (if seed does not form the reaction can be self-seeded by removing an aliquot, cooling to room temperature, and returning the crystals which formed to the bulk solution). The seedbed was allowed to develop for 1−2 h at 70−75 °C. The slurry was then cooled at 15 deg/h to room temperature. Prior to isolation of the product the internal temperature was lowered to 4 °C and the slurry was aged for ∼1 h at this temperature prior to filtration. The wet cake was washed with 2 × 8 L of *n*-heptane then dried on the filter under nitrogen overnight to give a 83% yield of **15** from **23** (9.02 kg, 94 wt %, 26.1 mol).[37] HPLC assay conditions: C-8 column (250 mm × 4.6 mm i.d., 5 μm particle size), 50% MeCN: 50% 0.1% v 60% $HClO_4$/H_2O mobile phase, ramp to 70% MeCN over 20 min, 1.5 mL/min at 35 °C and detecting at 210 nm wavelength.

2-*C*-Methyl-3,5-di-*O*-(4-methylbenzoyl)-D-ribofuranose (28). To a solution of 3-*O*-benzyl-1,2-*O*-isopropylidene-3-*C*-methyl-α-D-allofuranose (**15**) (5.0 kg, 15.4 mol) and pyridine (3.7 kg, 46.2 mol) in 35 L of acetonitrile was added 4-methylbenzoyl chloride (*p*-toluoyl chloride) (5.2 kg, 33.9 mol), and the reaction was heated at 50−55 °C for 12 h. A solution of 6.0 L (46.2 mol) of 48 wt % of HBF_4 in 9 L of water was added at 50−55 °C. After 2 h, 10 L of acetonitrile was distilled off under reduced pressure and 10 L of acetonitrile was added. At 97% conversion, 10 L of acetonitrile was distilled off, and the reaction solution was cooled to 0−5 °C. A solution of periodic acid (4.2 kg, 18.5 mol) in 10 L of water was added. After the reaction was aged for 30 min, 35 L of isopropyl acetate and 10 L of water were added. The organic phase was washed with 25 L of water followed by 20 L of aqueous $NaHCO_3$, 15 L of 5% sodium thiosulfate in water, and 15 L of water. The isopropyl acetate solution was concentrated to 10−15 L, and 40 L of methanol was added. The solution was cooled to 0 °C and diisopropylamine (0.78 kg, 7.7 mol) was added. After 2 d at 0 °C, aqueous HCl (1 N, 7.7 L) was added at 0−5 °C followed by 30 L of isopropyl acetate and 40 L of water. The organic phase was washed with aqueous 1 N HCl, $NaHCO_3$, and brine. The organic phase was dried through azeotropic distillation and treated with activated carbon. The carbon was removed by filtration and the resulting solution was diluted to 75 L with isopropyl acetate and hydrogenated (45 psi, 50 °C, 1.5 kg of 10% Pd/C) for 24 h. The filtrate was concentrated to 15 L, and 60 L of heptane was added at 50 °C. The crystalline product was isolated by filtration washing with a 10 L of 20% isopropyl acetate−heptane. Drying afforded 4.03 kg of the desired diol **28**. ^{1}H NMR ($CDCl_3$, 400 MHz): The ratio of α:β isomers in $CDCl_3$ was about 5 to 1. For the major isomer: δ 7.95−7.90 (m, 4H), 7.26 (d, J = 8.0 Hz, 2 H), 7.17 (d, J = 8.0 Hz, 2 H), 5.53 (d, J = 7.2 Hz, 1 H), 5.22 (d, J = 2.8 Hz, 1 H), 4.65−4.49 (m, 3 H), 3.08 (d, J = 3.2 Hz, 1 H), 2.44 (s, 3 H), 2.38 (s, 3 H), 2.26 (s, 1 H), 1.44 (s, 3 H). For the minor isomer: δ 7.95−7.90 (m, 4H), 7.27 (d, J = 8.0 Hz, 2 H), 7.22 (d, J = 8.0 Hz, 2 H), 5.16 (d, J = 5.6 Hz, 1 H), 5.12 (d, J = 5.6 Hz, 1 H), 4.66−4.49 (m, 3 H), 3.54 (d, J = 5.6 Hz, 1 H), 2.91 (s, 1 H), 2.43 (s, 3 H), 2.40 (s, 3 H), 1.44 (s, 3 H). ^{13}C NMR

(36) ^{1}H NMR data for the ketone has been reported. Soler, T.; Bachki, A.; Falvello, L. R.; Foubelo, F.; Yus, M. *Tetrahedron*: *Asymmetry* **2000**, *11*, 493. ^{1}H NMR data for the hydrate have been reported: see ref 21.

(37) The ^{1}H NMR data obtained for this compound agreed with published data. Dequin, B.; Bertounesque, E.; Guadel, G.; Florent, J.-C.; Monneret, C. *Tetrahedron* **1992**, *48*, 4885−4892.

(CDCl3, 100 MHz): δ 166.6, 166.3, 165.9, 165.7, 144.6, 144.3, 143.8, 143.7, 129.9, 129.7, 129.3, 129.2, 129.1, 129.0, 127.0, 126.9, 126.4, 126.2, 102.9, 100.8, 79.8, 79.2, 78.7, 76.9, 76.5, 76.4, 65.5, 64.0, 23.5, 21.7, 21.6, 20.0. Anal. Calcd for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04. Found: C, 65.59; H, 6.03.

1,2-Anhydro-3,5-di-*O*-(4-methylbenzoyl)-2-*C*-methyl-*β*-D-ribofuranose (29). To a 72-L vessel was charged dry dichloromethane (32 L), triethylamine (3.0 L), and diol **28** (3.44 kg). The mixture was warmed to 30 °C, then methanesulfonyl chloride (0.79 L) was added over 40 min. After 1 h, the batch was partitioned between pH 7 buffer (20 L) and methyl *tert*-butyl ether (44 L). The organic phase was washed with 1 M aqueous NaCl (38 L) then switched to toluene by vacuum distillation. The resulting solution of epoxide **29** (2.75 assay kg, 93%) was used directly in the subsequent glycosidation reaction. The HPLC method was same as that for **28**.

4-Phthalimido-7-[3′,5′-di-*O*-(4-methylbenzoyl)-2′-*C*-methyl-*β*-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (38). Tetrahydrofuran (5.4 L), sodium hydride (146 g of 60% dispersion in oil, unwashed), and *N*,*N*-dimethylacetamide (4 L) were charged to a 72-L flask and the suspension was cooled to 0 °C. 4-Phthalimido-7*H*-pyrrolo[2,3-*d*]pyrimidine (**37**) (2.08 kg) was added to the reaction while maintaining the temperature below 25 °C (Caution! Gas evolution). *N*,*N*-Dimethylacetamide (1.4 L) was added followed by a solution of the epoxide **29** (2.75 assay kg) in toluene (5.5 L), and the reaction mixture was heated at 50 °C for 9 h. After the solution was cooled to ambient temperature, ethyl acetate (19 L) and aqueous KH_2PO_4 (0.97 kg in 19 L of water) were added. The organic phase was washed with water. The resulting organic solution was solvent switched to toluene by vacuum distillation (<40 °C) to give a final volume of 10 L. The HPLC method was the same as that for **28**.

4-Amino-7-(2′-*C*-methyl-*β*-D-ribofuranosyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidine (1). To the toluene solution of **38** at ambient temperature was added methanol (25 L) and *n*-butylamine (3.13 kg, 42.87 mol). The reaction mixture was aged at 64 °C for 24 to 30 h. The mixture was concentrated to about 9 L, and 9 L of methanol was added. The solution was concentrated to 9 L, and the resulting slurry was aged at 60 °C for 60 min. Toluene (13 L) was added over 1.5 h. The slurry was aged at 60 °C for another 2 h and then allowed to cool to ambient temperature. The solid was filtered and the wet cake was washed with 9 L of 20% methanol in toluene then 9 L of 10% methanol in toluene. The solid was suction dried at ambient temperature under nitrogen to give 1.79 kg of **1** as the toluene solvate; the yield was 77% for the three steps from diol **28**. The nonsolvate form is available by two methods.

(a) Recrystallization of 1: The toluene solvate form of nucleoside **1** (30 g) was dissolved in 240 mL of 25% water in 1-propanol at 50 °C and the resulting solution was seeded. The water concentration was reduced to 2% by azeotropic distillation at reduced pressure while maintaining the volume constant with the addition of 1-propanol. The slurry was cooled to about 20 °C and the crystallized product was isolated by filtration to give 25 g of solid after suction drying at ambient temperature.

(b) Hot Slurry of 1 with 1-propanol: The toluene solvate form of nucleoside **1** (30 g) was dissolved in 240 mL of 2% water in 1-propanol and the slurry was heated at 60−65 °C for 4 h. After the solution was cooled to about 20 °C, the crystalline solid was isolated by filtration to give 25 g of the nucleoside as the unsolvated crystal form.

Analytical data for the nonsolvated **1**:[5] $[\alpha]_{365}$ −156.8 (MeOH, *c* 1.0 w/v %); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 0.64 (s, 3 H), 3.64 (ddd, J = 3.4, 5.0, and 12.1 Hz, 1 H), 3.80 (ddd, J = 2.0, 5.0, and 12.1 Hz, 1 H), 3.83 (ddd, J = 2.0, 3.4, and 8.9 Hz, 1 H), 3.93 (dd, J = 7.2 and 8.9 Hz, 1 H), 5.02 (s, 1 H), 5.06 (d, J = 7.2 Hz, 1 H), 5.09 (t, J = 5.0 Hz, 1 H), 6.12 (s, 1 H), 6.55 (d, J = 3.5 Hz, 1 H), 6.98 (br s, 2 H), 7.45 (d, J = 3.5 Hz, 1 H), 8.04 (s, 1 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 19.59, 59.76, 72.05, 78.64, 82.07, 90.53, 99.47, 102.42, 121.30, 149.69, 151.64, 157.46. Anal. Calcd for $C_{12}H_{16}N_4O_4$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.44; H, 5.42; N, 20.00.

2-(2,2-Diethoxyethyl)malononitrile (30). To a 100-L flask equipped with mechanical stirrer, thermocouple, and nitrogen/vacuum inlet was added 33 L of DMF and potassium carbonate (12.6 kg, granular, 91.1 mol). Malononitrile (6.02 kg, 91.1 mol) was melted at 40 °C overnight and added to the K_2CO_3 slurry and the mixture was washed in with 3 L of DMF. A purple slurry resulted with mild exotherm to 41 °C. Bromoacetaldehyde diethyl acetal (9.00 kg, 45.7 mol) was added. The slurry was degassed three times (placed under vacuum and back-filled with nitrogen) and warmed to 50 °C then aged 17.5 h. GC analysis showed >99A% conversion. The batch was cooled to ambient temperature and mixed with 80 L of H_2O and 40 L of toluene in a 200-L extractor. After layer cut, the top organic layer was washed with 2 × 70 L of 5% brine. GC assay of the organic layer indicated 5.41 kg (65% yield) of **30**. The dialkylated product (**31**) was present at 11 GC area %. The crude solution was used directly for the next step. An analytical sample was obtained as an oil after column chromatography on silica gel.

GC sample preparation from the reaction: ∼1 mL of reaction mixture was quenched into 10 mL of water and 10 mL of toluene, the toluene layer was diluted 3 times for GC assay on an 14% cyanopropyl phenyl methyl capillary column, 15 m × 530 μm, 1.00 μm film thickness; initial temperature 50 °C; ramp 15 deg/min to 250 °C then hold for 4 min; split ratio 5:1; retention times, toluene 2.0 min, DMF 3.0 min, bromoacetaldehyde diethyl acetal 4.2 min, malononitrile 4.9 min, **30** at 7.9 min, and **31** at 10.9 min. For conversion calculations toluene, DMF, and malononitrile peak integrations were deleted. For yield assay, a weighed 1-mL sample is diluted to 50 mL with MTBE for quantitative assay.

Potassium 4,6-Diamino-5-(2,2-diethoxylethyl)pyrimidine-2-thiolate (32). The crude organic solution of **30** was charged to a 100-L flask and concentrated to ∼8 L, and the concentrate was flushed with 15 L of toluene. The concentrate was flushed with 18 L of anhydrous ethanol. GC showed <5 vol % of toluene. The concentrate was diluted to 34 L with anhydrous ethanol. Thiourea (2.60 kg, 34.2 mol) was added in one portion and the slurry cooled with an ice bath. Potassium *tert*-butoxide solid (3.84 kg, 34.2 mol) was added over 15 min with 5.5 L of ethanol rinse and the slurry exothermed to 43 °C. The flask was fitted with a reflux condenser and reaction was warmed to 78 °C. Upon reaching temperature the reaction first became homogeneous then, after a 1−2 h age at 78 °C, a slurry formed. The reaction was aged an additional 16.5 h at 78 °C at which point the slurry was sampled. The sample was diluted with H_2O prior to HPLC assay, which showed 7.9 area % thiourea remaining (typically 5−10 area %). The reaction mixture was diluted with 7.6 L more of ethanol and cooled to 21 °C over 90 min. After aging 1 h 15 min at 21 °C, the batch was filtered and the cake washed with 38 L of ethanol in three portions. The wet cake was dried under nitrogen flow with vacuum on the filter pot to give 8.70 kg of off-white solid **32**. HPLC analysis showed 95.6 area % and acid titration showed 93 wt % purity. Loss on drying was 6.2 wt %. The corrected yield was 85%. HPLC assay conditions: 3 mm × 3 cm C-8 column; temperature 35 °C; flow rate 2 mL/min; eluent 0.1% H_3PO_4/acetonitrile, hold 1 min at 100% H_3PO_4 then ramped to 75/25 acetonitrile/H_3PO_4 over 6 min; UV 210 nm; retention times, thiourea 0.23 min, **33** 0.7 min, **32** 2.6 min, and disulfide 3.0 min.

4-Amino-7*H*-pyrrolo[2,3-*d*]pyrimidine-2-thiol (33). To a 100-L flask equipped with a mechanical stirrer, thermocouple, and N_2 inlet were charged 40 L of H_2O and 11.75 L of 5 N HCl. The potassium salt **32** (7.50 kg, 23.5 mol) was added over 25 min with 7 L of H_2O rinse and the solution was warmed to 50 °C over 50 min. A slurry was formed on warming. The resulting slurry was sampled after 10 min at 50 °C and the sample was diluted with water prior to HPLC assay. Assay at this point showed complete consumption of

starting material. The reaction slurry was transferred to a cooled vessel charged with a mixture of 12 L of H_2O and 3.5 L of 10 N NaOH. Mild exotherm following reagent addition caused the reaction to warm to 42 °C. The resulting slurry was cooled to 22 °C and then adjusted to pH 7, aged 3 h (typically aged 1−3 h, aging longer than 3 h led to increasing amounts of disulfide impurity), and filtered. The filter cake was washed with 30 L of water followed by 36 L of acetonitrile and dried under nitrogen to give 3.95 kg of off-white solid **33**. HPLC indicated 98.6 area % purity. Acid titration and loss on drying indicated 90.5 wt % (100% yield). HPLC conditions were the same as for **32**. Retention times: thiourea 0.23 min, **33** 0.7 min, **32** 2.6 min, disulfide, 3.0 min.

4-Amino-7*H*-pyrrolo[2,3-*d*]pyrimidine-2-sulfinic Acid (34). In a 100-L cylindrical vessel with heating/cooling coils, **33** (21.8 mol) was dissolved in 43.8 L of 1 N KOH and cooled to 5 °C. Hydrogen peroxide, 30% solution (4.72 kg, 43.6 mol), was added over 1.5 h, maintaining the reaction temperature at 5−15 °C. (Note: The addition was done in shots at ∼150−200 mL/min and the batch temperature was <13 °C during the addition.) The resulting solution was aged at 10 °C for 1 h then warmed to 15 °C. HPLC assay indicated 98% conversion. Acetic acid (6.8 L) was added over 20 min, resulting in the formation of a thick slurry. The reaction was then heated to 35−40 °C over 30 min, aged for 10 min, then cooled to 10 °C and aged for 20 min. The temperature cycle was done to increase the particle size to give a filterable solid. The crystalline solid was isolated by filtration and the resulting filter cake was washed with water (3 × 8 L) then acetone (8 L). The cake was dried on the filter under nitrogen to give 4.24 kg (99.1% yield, 92.5 area%) of sulfinic acid **34** as a pale yellow solid. HPLC conditions: C-8 column, 4.6 mm × 7.5 cm. Mobile phase: (A) 0.1% H_3PO_4; (B) acetonitrile. Gradient: 100:0 A/B hold 4 min to 50:50 A/B over 6 min. Flow: 1.5 mL/min. UV: 210 nm. Typical retention times: 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimine-2-sulfonic acid 2.1 min, **33** 2.7 min, **34** 3.6 min. ^{1}H NMR (DMSO-d_6, KOD in D_2O, 400 MHz): δ 7.04 (s, 1H), 6.21 (s, 1H). ^{13}C NMR (DMSO-d_6, KOD in D_2O, 100 MHz): δ 168.8, 158.4, 156.5, 135.8, 104.9, 96.4. Anal. Calcd for $C_6H_6N_4O_2S$: C, 36.36; H, 3.05; N, 28.27. Found: C, 36.13; H, 2.93; N, 27.95.

4-Amino-7*H*-pyrrolo[2,3-*d*]pyrimidine Hydrogen Sulfate (35). To a 100-L flask with nitrogen sweep to a caustic scrubber, the sulfinic acid **34** was added portion-wise to 56.7 kg of 50 wt % sulfuric acid at 20−30 °C over 1.5 h. The reaction was aged at 20−30 °C for 1 h. Gas evolution occurred during the addition and age. The reaction was then heated to 70−75 °C and aged 3 h, until dissolution was complete. (Note: The hot age converts the residual 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimine-2-sulfonic acid impurity to 4-amino-7*H*-pyrrolo[2,3-*d*]-pyrimidin-2-ol, which is more easily rejected in the crystallization. The sulfonic acid was undetected by HPLC after the hot age.) The reaction was then cooled to 15−20 °C. Ethanol was added over 45 min maintaining the temperature at <25 °C. The slurry was cooled to 10 °C and aged 20 min. The solid was isolated by filtration. The cake was washed with 20 L of cold 1:1 EtOH/50% sulfuric acid then with ethanol (4 × 8 L). The solid was dried on the filter under nitrogen giving 7.5 kg (84% yield, 98.3 wt %) of **35**. The HPLC conditions were the same as those for **34**. Typical retention times: 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-ol 1.8 min, **35** 2.2 min, **34** 3.6 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.61 (s, 1H), 9.14 (br s, 1 H), 8.52 (br s, 1H), 8.27 (s, 1H), 7.36 (dd, J = 3.3, 2.5 Hz, 1H), 6.87 (dd, J = 3.3, 1.8 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 151.4, 148.1, 142.4, 125.3, 102.6, 101.6. Anal. Calcd for $C_6H_8N_4O_4S$: C, 31.03; H, 3.47; N, 24.13. Found: C, 30.76; H, 3.17; N, 23.92.

***N*-(7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-yl)benzamide (36).** Acetonitrile (100 mL), brine (50 mL), and 10 N NaOH (20 mL) were combined in a 500-mL round-bottom flask. The flask was degassed by two vacuum/nitrogen fill cycles. To the vigorously stirred biphasic mixture was added 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine hydrogen sulfate (**35**) (26.58 g, 87.4 wt %, 100 mmol) at room temperature. The slurry was cooled to 15 °C and benzoyl chloride (46 mL, 396 mmol) was added dropwise with slight exotherm to 20 °C. With the mixture at 10−15 °C, 10 N NaOH (40 mL) was added dropwise over 25 min, resulting in a clearly biphasic mixture. The reaction was aged for 3 h with gradual warming to room temperature. The mixture was cooled to 5−10 °C and 10 N NaOH (40 mL) was added dropwise over 1 h. The reaction was warmed to room temperature and the reaction monitored by HPLC until hydrolysis was complete in 2 h. During this time, the product began to precipitate at the interface. Ammonium acetate (7.67 g, 99 mmol) was added to bring the pH of the aqueous layer to 10.2. The solid was isolated by filtration and washed with water (3 × 100 mL), acetonitrile (2 × 100 mL), and ethanol (50 mL). The cake was dried on the filter under nitrogen to give a 92% yield of benzamide **36** (21.92 g, 96.6 wt %). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.05 (br s, 1H), 10.97 (br s, 1H), 8.53 (s, 1H), 8.04 (d, J = 7.4, 2H), 7.58 (t, J = 7.3 Hz, 1H), 7.50 (t, J = 7.4 Hz, 2H), 7.40 (d, J = 3.3 Hz, 1H), 6.59 (d, J = 3.3 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 166.2, 153.7, 152.1, 151.3, 150.6, 134.2, 132.7, 128.9, 124.9, 109.3, 102.9.

2-(7*H*-Pyrrolo[2,3-*d*]pyrimidin4-yl)isoindoline-1,3-dione (37). In a 72-L flask, **35** (5.79 kg, 24.9 mol) and phthalic anhydride (7.46 kg, 50.3 mol) were added to 25 L of *N*,*N*-dimethylacetamide. The mixture was degassed to nitrogen 3 times then heated to 70−75 °C. *N*,*N*-Diisopropylethylamine (9.8 L, 55.2 mol) was added at a rate such that the batch temperature was maintained at <85 °C. Following addition, the resulting solution was aged at 80−85 °C for 5 h, then allowed to cool to room temperature. The conversion as determine by HPLC was 97%. The reaction was then further cooled to 12 °C with an ice bath. Water (12.5 L) was added to the resulting slurry over 1.5 h, maintaining the batch temperature at <25 °C. The slurry was aged for 1 h after addition at 12−15 °C. The solid product was isolated by filtration and the resulting filter cake was washed with 16 L of 1:1 *N*,*N*-dimethylacetamide/water, then 3 × 16 L of water. After some drying, the cake was washed with 12 L of ethyl acetate. The cake was dried on the filter under nitrogen to remove the bulk of the water, then dried at 60 °C in the vacuum oven to a water content of <0.2 wt % as determined by Karl−Fisher titration. The product **37** was isolated as an off-white solid in 85.6% yield (5.6 kg, 99.7 area % purity). The HPLC conditions were the same as those for **34**. Typical retention times: hydrogen sulfate salt 2.2 min, phthalic acid 6.7 min, amino deazapurine phthalimide 7.5 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.14 (s, 1H), 8.79 (s, 1H), 8.01 (dd, J = 5.5, 3.1 Hz, 2H), 7.92 (dd, J = 5.5, 3.1 Hz, 2H), 7.63 (dd, J = 3.5, 2.4 Hz, 1H), 6.56 (dd, J = 3.5, 1.8 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 166.3, 154.2, 151.0, 145.2, 135.7, 132.0, 128.7, 124.4, 115.2, 100.1. Anal. Calcd for $C_{14}H_8N_4O_2$: C, 63.64; H, 3.05; N, 21.20. Found: C, 63.33; H, 2.74; N 21.26.

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