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Lecture Transcripts

Independent Synthesis and Fate Studies of Impurities in Process Intermediates of the Anti-AIDS Drug d4T

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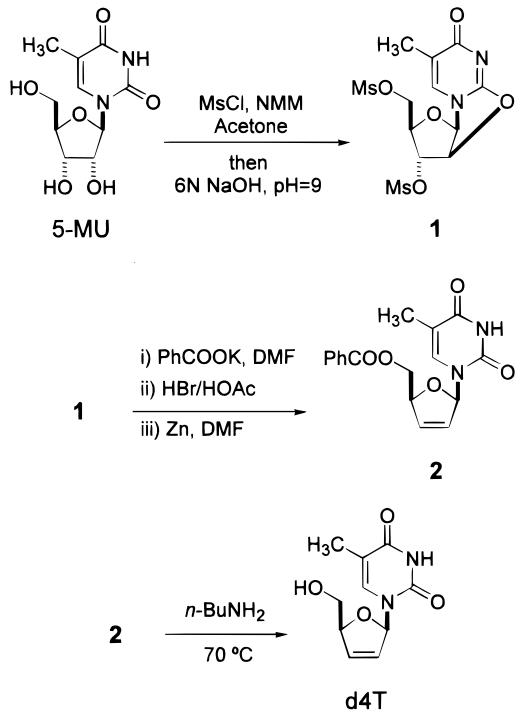
Abstract:

Impurities in isolated intermediates in a process to prepare d4T were identified, independently synthesized, and then taken through the process to determine their ultimate fate. Some of the products from these fate studies were also independently synthesized and used in the validation of impurity assay methods.

Introduction

d4T (stavudine, Zerit) is currently being marketed for the treatment of AIDS in the United States and other countries. Several syntheses of d4T have appeared in the literature.[1,2] Among them is one route that was developed by us recently for the production of d4T from 5-methyluridine (5-MU).[2] During the initial scale-up of this new process (Scheme 1), the levels of some impurities in isolated intermediates were observed to increase. Investigations into the causes of the higher levels revealed that these increases were generally due to the longer processing times that were required on larger scale, although in some cases there were other contributing factors. Independent synthesis of the impurities not only confirmed the structure assignments but also provided samples to carry out fate studies. In addition, samples of observed impurities and potential impurities were used to validate impurity assay methods.

Scheme 1



Results and Discussion

The only significant process impurity in the first step (5-MU → **1**, Scheme 1) of this synthesis is 5′-chloro-5′-deoxy-3′-*O*-(methylsulfonyl)-2,2′-anhydro-5-methyluridine, **3** (see eq 1), which is presumably formed from chloride displacement of the primary methylsulfonyl ester at C-5′ during the reaction. Independent synthesis of chloride **3** was easily accomplished by reaction of **1** with lithium chloride in DMF

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(1) For recent syntheses of d4T see: (a) McDonald, F. E.; Gleason, M. M. *J. Am. Chem. Soc.* **1996**, *118*, 6648. (b) Clive, D. L. J.; Wickens, P. L.; Sgarbi, P. W. M. *J. Org. Chem.* **1996**, *61*, 7426. (c) Mustafin, A. G.; Gataullin, R. R.; Spirikhin, L. V.; Abdrakhmanov, I. B.; Tolstikov, G. A. *Zh. Org. Khim.* **1996**, *32*, 1842. (d) Shiragami, H.; Ineyama, T.; Uchida, Y.; Izawa, K. *Nucleosides Nucleotides* **1996**, *15*, 47. (e) Becouarn, S.; Czernecki, S.; Valery, J.-M. *Nucleosides Nucleotides* **1995**, *14*, 1227. (f) Larsen, E.; Kofoed, T.; Pedersen, E. B. *Synthesis* **1995**, 1121. (g) Niihata, S.; Kuno, H.; Ebata, T.; Matsushita, H. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 2327. (h) Lipshutz, B. H.; Stevens, K. L.; Lowe, R. F. *Tetrahedron Lett.* **1995**, *36*, 2711. (i) Luzzio, F. A.; Menes, M. E. *J. Org. Chem.* **1994**, *59*, 7267. (j) Jung, M. E.; Gardiner, J. M. *Tetrahedron Lett.* **1992**, *33*, 3841. (k) Vargeese, C.; Abushanab, E. *Nucleosides Nucleotides* **1992**, *11*, 1549. See also ref 2 and references therein.

(2) Chen, B.-C.; Quinlan, S. L.; Stark, D. R.; Reid, J. G.; Audia, V. H.; George, J. G.; Eisenreich, E.; Brundidge, S. P.; Racha, S.; Spector, R. H. *Tetrahedron Lett.* **1995**, *36*, 7957.

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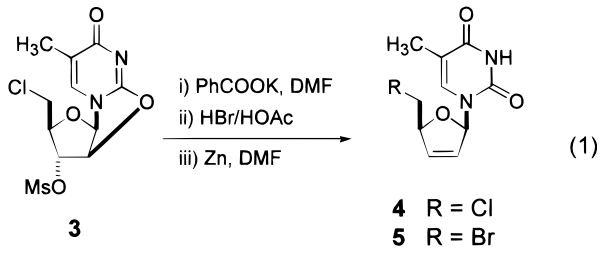
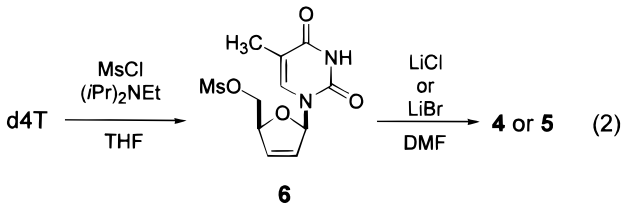
at 87 °C in 68% yield.[3]



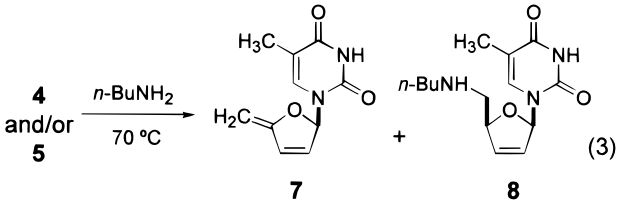
(1)

Since only a small quantity of **3** was removed during the isolation of anhydro-intermediate **1**, we were anxious to find out the fate of **3** during the remainder of the process. Subjecting **3** to the conditions of the second step of the process shown in Scheme 1 produced two new impurities, 5′-chloro-2′,3′-didehydro-3′,5′-dideoxythymidine, **4**, and the corresponding 5′-bromo analogue, **5**, as a 4:1 mixture of products in 78% yield (eq 1). Close examination of HPLC chromatograms of benzoyl d4T, **2**, that had been prepared from **1** containing appreciable amounts of 5′-chloro impurity **3** revealed the presence of **4** and **5**, albeit in reduced amounts. Pure samples of **4** and **5** were prepared independently in 84% and 72% yield, respectively, by reaction of the mesylate of d4T, **6** (eq 2), with lithium chloride or lithium bromide in DMF at 80 °C. Mesylate **6** in turn was prepared in 87% yield from d4T (MsCl, *i*-Pr_2NEt, THF, 0 °C → rt).



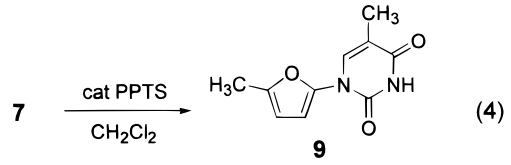
(2)

Treating **4** and/or **5** with *n*-butylamine, as in the third step of the process (**2** → d4T), provided **7** and **8** (HPLC ratio 9:1), formal products of β-elimination and displacement, respectively, as a mixture that was separable by flash column chromatography (eq 3).[4]



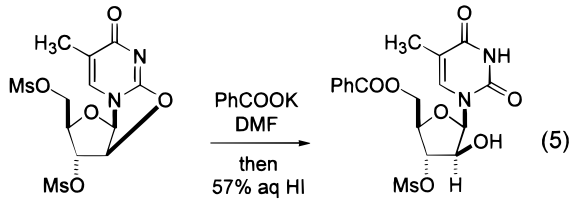
(3)

Unacceptably high levels of exo-olefin **7** and *n*-butylamino derivative **8** were observed in some laboratory lots of d4T, which were prepared from lots of benzoyl d4T, **2**, that contained high levels of impurities **4** and **5** (both sources of **7** and **8**). Since **7** and **8** were not consistently removed during the isolation of d4T, an optional rework procedure was developed for benzoyl d4T, **2**, a highly crystalline late intermediate from the previous step. Eventually, a recrystallization from CH_3OH was developed that provided pure benzoyl d4T, **2**, with complete removal of impurities **4** and **5**.



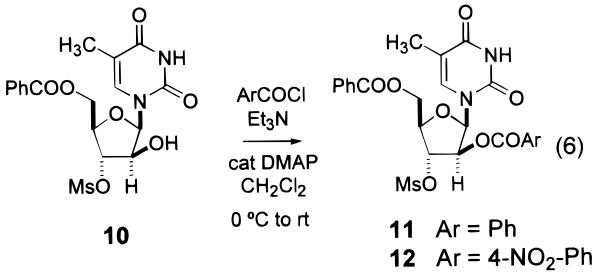
(4)

While exo-olefin **7** could not be effectively removed from d4T directly, we reasoned that, with acid catalysis, **7** could be converted to methylfuran **9** (eq 4), which might prove easier to remove. Thus, treatment of **7** with 0.15 equiv of PPTS in CH_2Cl_2 at room temperature gave **9** in 95% yield after flash chromatography. Unfortunately, d4T is itself sensitive to acid, and no effective purification procedure utilizing an acid treatment was found for d4T contaminated with **7**.



(5)

Impurity **10** (eq 5) was isolated from a batch of benzoyl d4T, **2**, that was produced on large scale. Its preparation from **1** is shown in eq 5. Treatment of **1** with PhCOOK in hot DMF followed by 57% aqueous HI gave **10** in 59% yield. To determine the configuration of the C-2′ hydroxyl, **10** was converted to **11** (eq 6). A comparison of NOE enhancements of **11** and dibenzoate **13** (see Figure 1), which had been isolated from an impure laboratory lot of **2**, was then made to allow the assignment of stereochemistry.



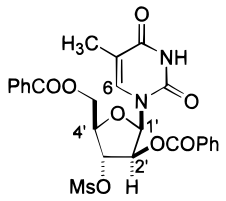
(6)

Figure 1 summarizes the results from the $^1H-^1H$ NOE experiments performed on **11** and **13**.[5] Irradiation of $H_{1'}$ in **11** resulted in a 9.4% enhancement of $H_{2'}$ and a 2% enhancement of the signal due to $H_{4'}$. In addition, irradiation

(3) None of the impurity syntheses described in this publication are optimized. All new compounds were characterized by $^1H/^{13}C$ NMR, IR, UV and MS. We thank Drs. H. Y. Lin (CDAS) and D. W. Dodsworth (AR&D) for gathering MS data.

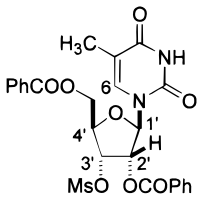
(4) Interestingly, treatment of mesylate **6** directly with *n*-butylamine gave **8** as the major product (73% HPLC area) along with **7** (12% HPLC area) and d4T (8% HPLC area). Also, reaction of **6** with bases such as *i*-Pr_2NEt and *i*-Pr_2NH gave only decomposition.

(5) All ^1H and ^{13}C chemical shift assignments were made from a combination of several NMR experiments (DEPT, COSY, HMQC, HMBC) performed on a Bruker DRX 360 MHz or a Bruker DRX 400 MHz spectrometer in DMSO-d_6 as solvent. The NMR spectra of **18** and **19** were obtained in CD_2Cl_2.



11

Signal Irradiated	Observed Enhancement
$H_{1'}$ (6.48 ppm)	$H_{2'}$ (9.4%), $H_{4'}$ (4.55 ppm, 2%)
$H_{2'}$ (5.82 ppm)	$H_{1'}$ (9.3%), $H_{4'}$ (1.6%)



13

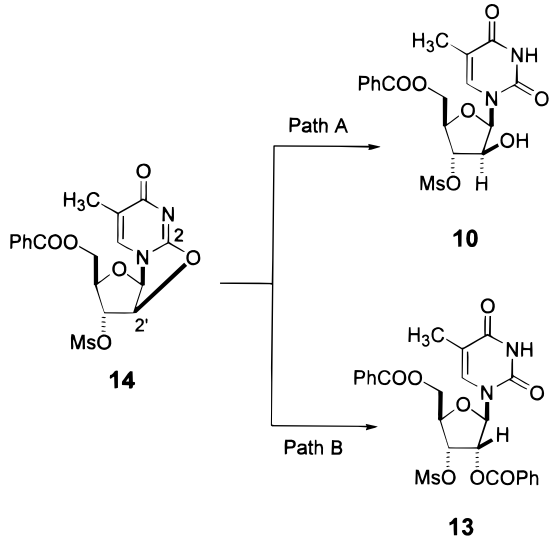
Signal Irradiated	Observed Enhancement
$H_{1'}$ (6.1 ppm)	$H_{2'}$ (1.7%), $H_{4'}$ (4.7 ppm, 3.1%) H_6 (3.5%)
$H_{2'}$ (5.85 ppm)	H_6 (4.9%), $H_{1'}$ (2.7%), $H_{3'}$ (6.7%)
$H_{3'}$ (5.63 ppm)	$H_{2'}$ (6.4%)
H_6 (7.5 ppm)	$H_{1'}$ (6.6%), $H_{2'}$ (5.8%)

Figure 1. **Results from NOE experiments on 11 and 13.**

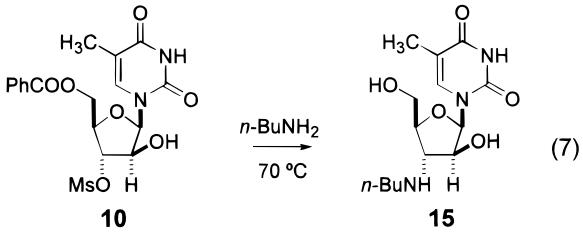
of $H_{2'}$ in **11** gave enhancements of 9.3% and 1.6% of the $H_{1'}$ and $H_{4'}$ signals, respectively. These results suggest a cis substitution pattern across C-1′,2′ in **11**. On the other hand, irradiation of $H_{1'}$ in **13** gave enhancements of the $H_{2'}$, $H_{4'}$, and H_6 signals of 1.7%, 3.1%, and 3.5%, respectively. Enhancements of the signals due to H_6 (4.9%), $H_{1'}$ (2.7%), and $H_{3'}$ (6.7%) were noted when $H_{2'}$ was irradiated. When signal $H_{3'}$ was irradiated, an enhancement of 6.4% was observed on $H_{2'}$. These results are consistent with the stereochemistry assigned to C-2′ in **13**. Further confirmation of the stereochemistry of **10** came from X-ray crystallographic analysis of the *p*-nitrobenzoate derivative, **12**, which was prepared from **10** in 75% yield (eq 6).

It is interesting to note that while **10** and **13** are formed as impurities during the same reaction (**1** → **2**, Scheme 1), their configuration at C-2′ is opposite. This can be explained by the involvement of the common intermediate, **14** (Scheme 2), which is formed from **1** in the d4T process. Anhydro-intermediate **14** can undergo acid-catalyzed hydrolysis at C-2 by adventitious water to yield **10** (Scheme 2, path A), while displacement by PhCOOK at C-2′ can yield **13** (path B). It should be noted that 2′*β*-benzoate **11** was not observed as an in-process impurity or as an impurity in the isolated intermediate, benzoyl d4T, **2**. It was synthetically prepared from **10** as described above.

Scheme 2



While we were pleased to find out that **10** was also completely removed during the optional CH_3OH recrystallization of **2**, we were interested in determining its fate through the rest of the process. Thus, reaction of **10** with *n*-$BuNH_2$ at 70 °C (eq 7) gave a major product identified as **15**, among other unisolable substances.



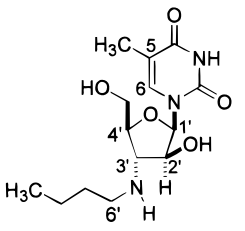
(7)

The configurations at C-2′ and C-3′ of **15** were determined from $^1H-^1H$ NOE experiments summarized in Figure 2.[5] Thus, irradiation of $H_{1'}$ resulted in an enhancement of 6.6% of $H_{2'}$ and 0.9% of H_6, while irradiation of $H_{2'}$ gave enhancements of 13.7%, 8.3%, and 1.8% of the signals due to $H_{1'}$, $H_{2'-OH}$, and $H_{3'}$, respectively. Irradiation of $H_{3'}$ caused enhancement of signals from H_6 (4.2%), $H_{2'-OH}$ (1.5%), and $H_{2'}$ (2%). In addition, irradiation of $H_{6'}$ (CH_2 next to N on the *n*-butyl chain) gave enhancement of 4.1% and 4.4% of the $H_{2'}$ and $H_{3'}$ signals, respectively.

Impurity **15** is likely formed by debenzoylation of **10** with concomitant formation of a 2′,3′-*β*-epoxide, which in turn could be opened by excess *n*-butylamine present in the reaction.[6] Fortunately, **15** and other by-products from this reaction are completely removed during the isolation of d4T.

Another impurity present in some batches of **2** was isolated and initially identified as **18** (Scheme 3). Furan **18** was prepared from **7** in two steps as shown in Scheme 3. Epoxidation of the exocyclic double bond in **7** presumably gave the corresponding epoxide, which isomerized readily

(6) Only one regioisomer was isolated from the ring opening of the putative 2′,3′-*β*-epoxide intermediate by *n*-butylamine. It is not clear why there is a preference for the formation of **15** over the other regioisomer. More likely, a minor regioisomer was formed in the reaction and it was one of the unisolable substances (vide supra).

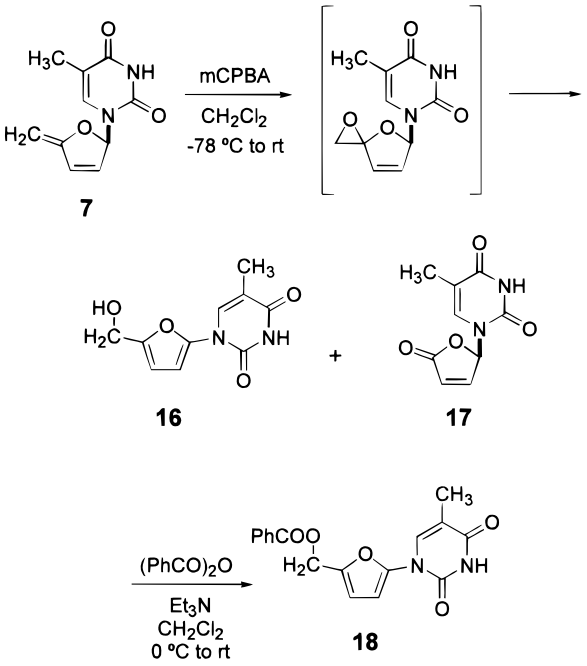


15

Signal Irradiated	Observed Enhancement
H_6 (7.53 ppm)	$H_{1'}$ (1.1%), $H_{2'\text{-OH}}$ (1.3%) $H_{3'}$ (2.2%), $H_{5\text{-Me}}$ (1.9%)
$H_{1'}$ (5.94 ppm)	H_6 (0.9%), $H_{2'}$ (6.6%)
$H_{2'\text{-OH}}$ (5.32 ppm)	H_6 (2.1%), $H_{2'}$ (8%), $H_{3'}$ (2%)
$H_{2'}$ (3.99 ppm)	$H_{1'}$ (13.7%), $H_{2'\text{-OH}}$ (8.3%), $H_{3'}$ (1.8%)
$H_{3'}$ (2.93 ppm)	H_6 (4.2%), $H_{2'\text{-OH}}$ (1.5%), $H_{3'}$ (2%)
$H_{6'}$ (2.56 ppm)	$H_{2'}$ (4.1%), $H_{3'}$ (4.4%)

Figure 2. **Results from NOE experiments on 15.**

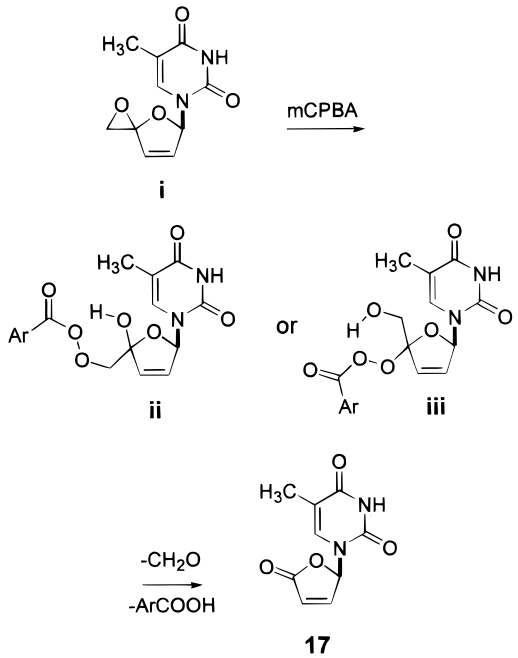
Scheme 3



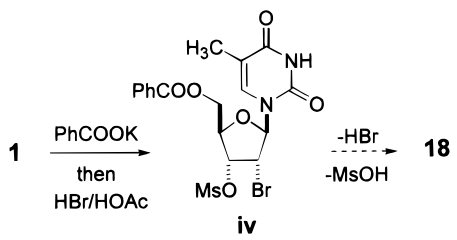
under the reaction conditions to provide **16** in 41% yield. Lactone **17**, which was difficult to separate by flash chromatography from **16**, was also formed during this reaction.[7] Benzoylation of (hydroxymethyl)furan **16** (still containing lactone **17**) provided benzoate **18** as expected. Compounds **17** and **18** were easily separated at this stage by flash chromatography. Upon examination of the ^{1}H and ^{13}C NMR spectra, as well as HPLC retention times and UV spectra, it was clear that the synthetic material, **18**, was different from the impurity that was isolated from **2**. The molecular weight of both these substances was found to be the same (326) as determined by LRMS.

The ^{1}H NMR[5] spectrum of the impurity indicated a methyl group (1.83 ppm, d, J = 1 Hz, 3H) which was coupled to a vinyl C*H* (7.14 ppm, d, J = 1 Hz, 1H). A broad singlet at 9.48 ppm (1H) indicated an N*H*, and multiplets at 8.17 (2H), 7.66 (1H), and 7.53 ppm (2H) indicated a phenyl group. Two doublets at 7.41 and 6.61 ppm (J = 2.3 Hz, 1H each) along with a singlet at 4.92 ppm were also present in the spectrum. The ^{13}C NMR[5] spectrum showed three carbonyl peaks at 163.9, 163.6, and 150.5 ppm. Ten more carbons, of which four were quaternary carbons (DEPT), were evident in the 100−150 ppm range. Signals at 11.7 ppm (CH_3) and 40.7 ppm (CH_2) completed the carbon NMR spectrum. On the basis of the molecular weight derived from the MS data, and the NMR spectra, we initially deduced the structure of the impurity to be **18**. However, two aspects of the NMR data troubled us about this structure. First, in the ^{1}H NMR spectrum, the doublets at 7.41 and 6.61 ppm were almost a full part per million apart, and second, in the ^{13}C NMR spectrum, the CH_2 was at 40.7 ppm (instead of the expected ∼60 ppm).[8] The NMR data from the synthetic sample confirmed our suspicion that our assigned structure may be wrong. While most other signals were comparable, the doublets at 6.61 and 6.48 ppm (J = 3.6 Hz, 1H each)

(7) The formation of lactone **17** may be explained by the mechanism shown below. Ring opening of the spiro epoxide **i** by *m*-CPBA could give rise to intermediate **ii** and/or **iii**. Fragmentation of **ii/iii** can then yield **17** from the formal loss of *m*-chlorobenzoic acid and formaldehyde.

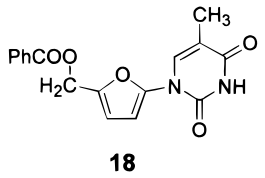


(8) Nevertheless, **18** made good sense in that we saw how this could be easily formed by elimination of the elements of HBr and methanesulfonic acid from **iv**, an intermediate in the preparation of **2** (resulting also in aromatization of the furan ring).

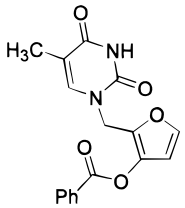


highlighted the difference in the ^{1}H NMR of the synthetic sample. Additionally, a CH_2 signal at 57.9 ppm was present in the ^{13}C NMR spectrum of the synthetic sample while all the other signals were comparable to the spectrum of the impurity sample.

We speculated then that the impurity may be **19** instead of **18**, purely on the basis of the NMR chemical shift data of the impurity. The identity of the isolated impurity was unequivocally established by X-ray crystallography to be **19**. The synthetic substance, on the other hand, was shown to be **18**, again by X-ray crystallography.



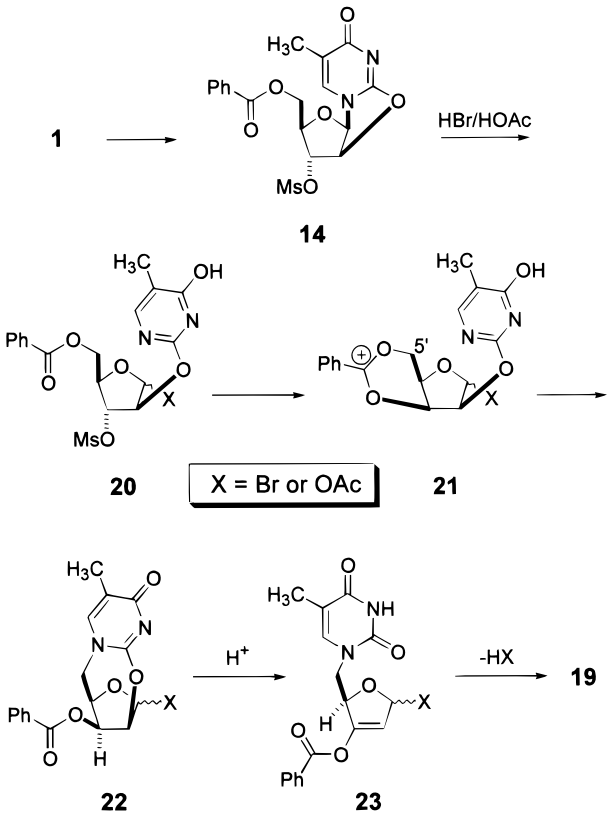
18



19

The formation of **19** during the preparation of **2** (from **1**) may be explained as shown in Scheme 4. Intermediates **20** (X = Br or OAc) could result from **14** by the reaction of HBr/HOAc in hot DMF. An equilibrium between **14** and **20** may even be possible. Neighboring-group participation with displacement of the C-3′ mesylate by the benzoate group at C-5′ could give the stabilized carbocation **21**. Bond formation across N-1 and C-5′ along with cleavage of the oxygen−C5′ bond would yield **22**. Protonation of the pyrimidine ring followed by elimination across C-2′ and C-3′ to give **23** and then a second elimination (of HX) would yield **19**.

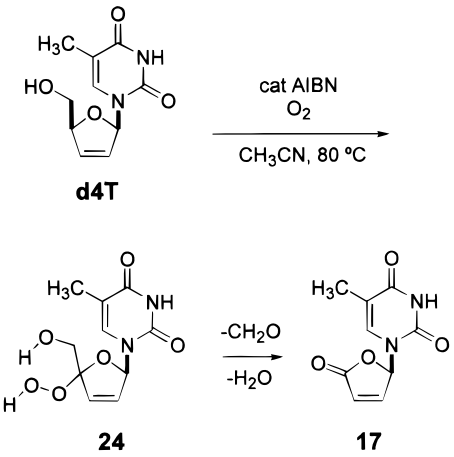
Scheme 4



As in the case of **10**, furan **19** is converted to products which are removed during the isolation of d4T in the subsequent step. It is also removed from **2** during the optional CH_3OH recrystallization. Exposure of a sample of **19** to the conditions of step 3 (**2** → d4T, Scheme 1) led to its conversion to a large number of minor products, none of which were identified.

An independent preparation of lactone **17** was achieved by bubbling oxygen through a hot solution of d4T in CH_3CN/*N*-methyl-2-pyrrolidinone in the presence of catalytic AIBN (Scheme 5) in 16% yield. This reaction is presumed to go through hydroperoxide **24**, which upon fragmentation would provide **17**.

Scheme 5



Conclusions

In conclusion, many impurities observed during the development of the 5-MU process for d4T were isolated and definitively identified. In addition, these impurities were synthesized independently, and fate studies were performed. Process modifications have been made in order to avoid the formation of these impurities, and this process now delivers very pure d4T in high overall yield.

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