Organic Process Research & Development **2000**, *4*, 172–174

New Synthesis of a Protected Ketonucleoside by a Non-Cryogenic Oxidation with TFAA/DMSO[1]

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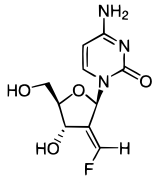
Abstract:

An improved synthesis of the ketonucleoside 2′-Oxo-3′,5′-*O*-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl]-cytidine (6), an intermediate in the synthesis of the potent anti-tumor agent 1 (MDL 101,731 or FMdC), is reported which incorporates a trifluoroacetic acid/dimethylsulfoxide oxidation process. This oxidation procedure eliminates the cryogenic reaction conditions and the necessity of protection at the N-4 amine used in the previously published route. Simplified isolation procedures for 3 and 4 eliminate two chromatographic purification steps. Overall yields are comparable to those reported previously.

Introduction

(*E*)-2′-Deoxy-2′-(fluoromethylene)cytidine (**1**)[3], a mechanism-based ribonucleotide reductase inhibitor, has shown broad-spectrum anti-tumor activity in preclinical studies.[4] This compound is currently being evaluated by Matrix Pharmaceutical, Inc. in Phase II clinical trials for non-small cell lung cancer and for colorectal cancer.

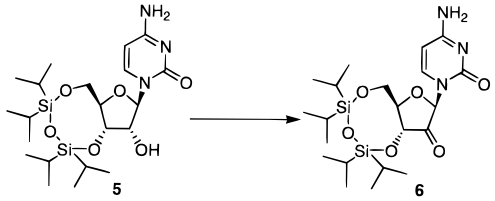


1
(MDL 101,731 or FMdC)

The original synthesis of **1** starting from uridine,[5] as well as an improved synthesis starting from cytidine,[6] has appeared in the literature. Amidine-protected ketoneucleoside **3**, an intermediate in the improved synthesis of **1**, was produced in the three-step, two-pot process shown in Scheme 1. The Swern's oxidation[7] step necessitated the use of cryogenic reaction temperatures (− 75 °C). However, the use of such low temperatures on large scale can be prohibitively expensive. In addition, unwanted hydrolysis of the N-4 amidine-protecting group in **3** was found to be an issue both during the reaction work-up/isolation process and during the storage of isolated solid **3**. This paper describes the development of a more practical oxidation method which eliminates the need for the N-4 amine protection and cryogenic reaction conditions.

Results and Discussion

A variety of oxidation procedures exist for the synthesis of ketonucleosides; however most have scale-up issues including low reaction temperatures (Swern's oxidations)[8] or waste concerns (CrO_3),[9] and often necessitate protection at N-4 (if present). For the synthesis of **1**, oxidation conditions were screened for the conversion of TIPDS-protected alcohol **5** to ketonucleoside **6**.



5 **6**

Alcohol **5** was synthesized starting from cytidine using procedure similar to that published previously.[10] Protection of the 3′- and 5′-hydroxyl groups in cytidine with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[11] (TIPS-Cl_2) in pyridine at room temperature gave complete conversion within 5 h. Dilution of the reaction mixture with hexane/water/ethyl acetate caused product precipitation. Alcohol **5** was isolated in essentially quantitative mass yield with HPLC purity of 94%. Recrystallization from *i*-propyl acetate did not significantly improve the purity of **5**. This solid was used for the subsequent oxidation screens without purification.

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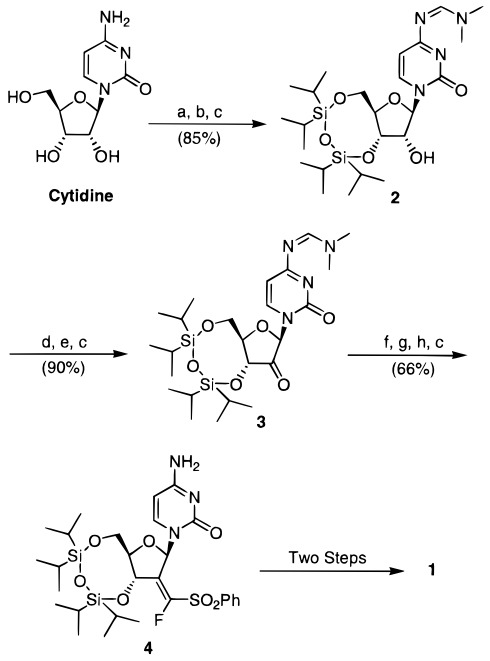
 10.1021/op9900939 CCC: $19.00
Published on Web 04/08/2000

Scheme 1. **Published route[6] to 1 via ketonucleoside 3[a]**



[a] Reagents and conditions: (a) TIPDS-Cl_2, pyridine, 22 °C; (b) dimethylformamide−dimethyl acetamide; (c) chromatography; (d) $(COCl)_2$, CH_2Cl_2, DMSO, −75 °C; (e) TEA, warm; (f) $PhSO_2Cl$, $(EtO)_2P(O)Cl$, LiHMDS, −70 °C; (g) aqueous NH_4Cl; (h) NH_3, MeOH, 22 °C.

Table 1. **Oxidation procedures for conversion of 5 to 6**

entry	oxidant	solvent	reaction temp (°C)	reaction time (hr)	HPLC area% conver.
1	oxalyl chloride/ DMSO	CH_2Cl_2	−50	0.5	0[a]
2	acetic anhydride/ DMSO	DMSO	19	17	0[a]
3	NaOCl	acetic acid	22	1	0[a]
4	NaOCl	ethyl acetate/ H_2O[b]	22	1	0[a]
5	$Al(OtBu)_3$/acetone	toluene	73	18	0[c]
6	5% TPAP/NMMO[d]	CH_2Cl_2	22	18	0[c]
7	P_2O_5/DMSO	CH_2Cl_2	22	18	9
8	TFAA/DMSO	pyridine[g]	−20	0.1	25[e]
9	TFAA/DMSO	pyridine/THF[g]	−20	1	100[e,f]
10	TFAA/DMSO	THF	−30	0.3	92
11	TFAA/DMSO	THF	−15	0.3	96
12	TFAA/DMSO	THF[h]	−15	0.3	95

[a] Decomposition of starting **5**. [b] Phase transfer catalyst used. [c] Starting **5** recovered. [d] Tetrapropylammonium perruthenate/*N*-methyl morpholine-*N*-oxide. [e] Significant side product formation. [f] No starting **5** remaining. [g] Using crude protection reaction mixture. [h] Doubled reaction concentration.

A variety of oxidation methods were investigated for the conversion of **5** to **6** (Table 1). Swern's oxidation at cryogenic temperatures (entry 1) failed to give the desired product, instead it produced side-products related to reaction at the unprotected N-4 nitrogen. Similar results were obtained using DMSO/acetic anhydride[12] (entry 2) or sodium hypochlorite[13] (entries 3 and 4). Oppenauer oxidation[14] (entry 5) or oxidation with catalytic perruthenate[15] (entry 6) gave only unreacted starting material. Oxidation with DMSO/P_2O_5[16] (entry 7) produced only small amounts of **6**, even after 18 h.

Results from the oxidation of **5** with DMSO/TFAA[17] were more encouraging. Treatment of the cytidine protection reaction mixture directly with DMSO and TFAA, followed by triethylamine, gave 25% conversion to desired **6** along with small amounts of side products related to trifluoroacetylation at the N-4 or the O-2′ positions (entry 8). Dilution of the protection reaction mixture with THF before the oxidation reaction and longer reaction times gave greater conversion but significant side reaction (Entry 9). When isolated **5** was used in the DMSO/TFAA oxidation procedure, 92 to 96% conversions were realized at reaction temperatures of −30 and −15 °C, respectively, with little formation of trifluoroacetylation side-products (entries 10 and 11). When the reaction concentration in entry 11 was doubled to 15 wt % alcohol **5** (entry 12), reaction conversion and selectivity were essentially identical.

For the synthesis of ketonucleoside **6** from isolated alcohol **5** by the use of this DMSO/TFAA process, TFAA (1.6 equiv) was added dropwise over 0.3 h to a solution of alcohol **5** in 6:1 THF/DMSO at −15 °C. After the resulting homogeneous mixture stirred at −15 °C for 0.5 h, the reaction was quenched by the addition of triethylamine (4 equiv). The reaction mixture was then warmed to room temperature, and the crude product was isolated by precipitation with water. The crude product (84 area% **6** by HPLC analysis) was purified by dissolving in ethyl acetate, washing with water, and reprecipitating by solvent exchange with toluene. This gave ketonucleoside **6** in 80−85% overall yield from starting alcohol **5** (not correcting for the purity of **5**) which was greater than 98 area% pure by HPLC.

Subsequent Horner-Emmons olefination of ketonucleoside **6** has been shown to give desired fluoro-olefin **4** with yields and purities comparable to those using N-4-protected ketonucleoside **3**. This demonstrates that N-4 protection is unnecessary for this process.

Conclusions

An improved synthesis of ketonucleoside **6**, an intermediate in the synthesis of the potent anti-tumor agent **1** (MDL 101,731 or FMdC), was developed which incorporated a noncryogenic TFAA/DMSO oxidation process. This oxidation procedure eliminated the cryogenic reaction conditions and the necessity of protection at the N-4 amine, used in the previously published route. Simplified isolation procedures for **5** and **6** eliminated two chromatographic purification steps. Overall yields of ketonucleoside **6** from cytidine were 80−85%, comparable to those reported previously for N-4-protected ketonucleoside **3**. Ketonucleoside **6** was subsequently shown to work as well as the N-4-protected ketonucleoside **3** in the overall process to produce **1**.

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Experimental Section

General. Cytidine (99%), TIPDS-Cl_2 (97%), trifluoroacetic anhydride (99+%), and triethylamine (99%) were purchased from Aldrich and used without purification. All solvents were ACS grade and were purchased from Fisher. Pyridine, DMSO, THF, and triethylamine were dried over 4Å molecular sieves.

^{1}H NMR spectra were collected by using a Bruker ACE-300 (300 MHz) spectrometer. Melting points were determined on a Thomas-Hoover Uni-Melt apparatus and are uncorrected. In-process and final product HPLC analysis was performed using two Zorbax Rx-C8 cartridge columns (4.0 × 80 mm each, 5 μm particle size) in series eluting with 70% aqueous acetonitrile (v/v) at 1.5 mL/min. and UV detection (235 nm).

3′,5′-*O*-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl]-cytidine (5). TIPDS-Cl_2 (68.51 g, 217 mmol) was added room temperature to a slurry of cytidine (50.14 g, 206 mmol) in dry pyridine (115.7 g) over 0.5 h with stirring. The internal temperature rose to 43 °C during the addition. The light yellow, homogeneous solution was cooled to room temperature, and the now hazy mixture stirred at that temperature for 5 h. HPLC analysis showed complete consumption of starting material. Attempts to precipitate the desired product by addition of hexane (220 mL) gave two liquid layers. This was poured into water (200 mL) and the flask rinsed with ethyl acetate (50 mL) and water (25 mL). When the original mixture and the rinses were combined, a precipitate formed. The precipitate was isolated by vacuum filtration and the filter cake washed with water (50 mL) and hexane (50 mL). The wet cake was dried in vacuo (25 Torr, 50 °C) leaving a white solid (99.41 g, 99.3% yield). HPLC analysis of the solid showed it to be 94 area% pure. This material matched the published ^{1}H NMR data[10] and was used without additional purification.

2′-Oxo-3′,5′-*O*-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl]-cytidine (6). Crude **5** (5.283 g, 94 area% pure by HPLC, 10.22 mmol) was suspended in dry THF (24.39 g). Dry DMSO was added dropwise until a homogeneous solution was achieved (4.261, 54.6 mmol), and the resulting solution was cooled to −15 °C. Trifluoroacetic anhydride (3.450 g, 16.43 mmol) was added dropwise over 0.25 h. No significant heat liberation was noted. The colorless, homogeneous solution was stirred at −15 °C for 0.5 h after which dry triethylamine (4.44 g, 43.9 mmol) was added over 0.1 h. During this addition, the reaction temperature rose from −15 °C to −10 °C. The pale yellow reaction mixture was warmed to room temperature and stirred at that temperature for 1 h. The mixture was then slowly added to cold water (100 mL) with vigorous stirring. The resulting precipitate was collected by suction filtration, and the filter cake was thoroughly washed with water (50 mL). The filter cake was dried in vacuo leaving a tan, free-flowing powder (5.23 g) that was 84 area% **6** by HPLC analysis. HPLC assay of this material showed 4.35 g of **6** in this solid (88% yield). Crude **6** can be purified as follows: The crude **6** was dissolved in ethyl acetate, and the resulting solution was washed with water. The organic phase was then solvent-exchanged to toluene and the resulting slurry filtered. The wetcake was rinsed with toluene and dried in vacuo (10 Torr, 40 °C), leaving a white solid. Recovery of **6** was typically 80−85% of material that was 98 area% pure by HPLC: mp 148−152 °C dec (lit.[18] mp 148−151 °C dec). This material matched the published ^{1}H NMR data[18] and was used without additional purification.

Acknowledgment

The authors thank Dr. Richard Donaldson, Mr. Neal Fetner (The Dow Chemical Company), and Dr. James McCarthy[19] (Hoechst Marion Roussel) for helpful discussions throughout the completion of this work.

Received for review October 21, 1999.

OP9900939

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