Supporting Material To:

Toward Orthopoxvirus Countermeasures: A Novel Heteromorphic Nucleoside of Unorthodox Structure

Xuesen Fan, Xinying Zhang, Longhu Zhou, Kathy A. Keith, Mark N. Prichard, Earl R. Kern, and Paul F. Torrence

Experimental Section

Melting points were recorded with a Barnstead 1201D electrothermal melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer. CD_3OD was used as solvent. The low resolution mass spectra (LRMS) were performed on a HP 1100 MSD spectrometer at the HT Laboratories, San Diego. The HRMS (High Resolution Mass Spectra) were performed on a JEOL HX 110A spectrometer at the Department of Chemistry, University of Arizona. Silica gel column chromatography was conducted with Sigma-Aldrich silica gel (70~230 mesh).

Experimental Procedure for the Preparation of 2.

Ethanol (6 mL) was added to a mixture of 5-formyl-2′-deoxyuridine (**1**, 128 mg, 0.5 mmol), malononitrile (39.6 mg, 0.6 mmol), and 1,3-cyclohexanedione (67.2 mg, 0.6 mmol). The mixture was stirred at 50°C overnight. Upon completion (checked by TLC using of 20% MeOH in $CHCl_3$), the mixture was allowed to cool to room temperature. The precipitate was collected by suction, rinsed with cold ethanol, and dried

to give compound **2.** The filtrate was concentrated under reduced pressure and the residue was purified through silica gel column chromatography ($CHCl_3$:MeOH, 5:1). The fractions containing the product were combined and concentrated to give the second batch of **2.** The total yield was 168 mg (81%) of **5-(2-amino-3-cyano-5-oxo-5,6,7,8-tetrahydro-4H-chromen-4-yl)-1-(2-deoxypentofuranosyl)-pyrimidine-2,4(1H,3H)-dione (2):** m.p. 216~218 °C; UV λmax = 275 nM (ε = 1.7 x 10^4); ^{1}H NMR (CD_3OD) δ: 1.98~2.03 (m, 2H, -CH_2, 7 H-chromene), 2.15~2.27 (m, 2H, -CH_2, 2′ H), 2.34~2.36 (m, 2H, -CH_2, 8 H-chromene), 2.56~2.61 (m, 2H,6-CH_2, 5 H-chromene), 3.74~3.81 (m, 2H, 5′-H), 3.92~3.94 (m, 1H, 4′ H), 4.17 (s, 1H, -CH, 4 H-chromene), 4.40~4.42 (m, 1H, 3′ H), 6.23~6.27 (m, 1H, 1′ H), 7.91 (s, 0.5H, 6 H, one isomer, 50%), 7.96 (s, 0.5H, 6 H, another isomer, 50%); ^{13}C NMR ($CDCl_3$) δ: 20.18, 26.81, 30.00, 30.07, 36.51, 36.54, 40.34, 40.43, 55.70, 55.99, 61.76, 61.87, 71.02, 71.18, 85.45, 85.52, 87.89, 87.96, 111.65, 114.86, 115.19, 119.73, 138.62, 138.96, 150.85, 160.31, 160.51, 163.08, 166.74, 166.84, 198.22, 198.25. ESI LRMS *m/e*: 417 (MH^+), 439 (MNa^+). HRMS (FAB) Calcd for $C_{19}H_{21}N_4O_7$: 417.1411 $(MH)^+$, found 417.1418.

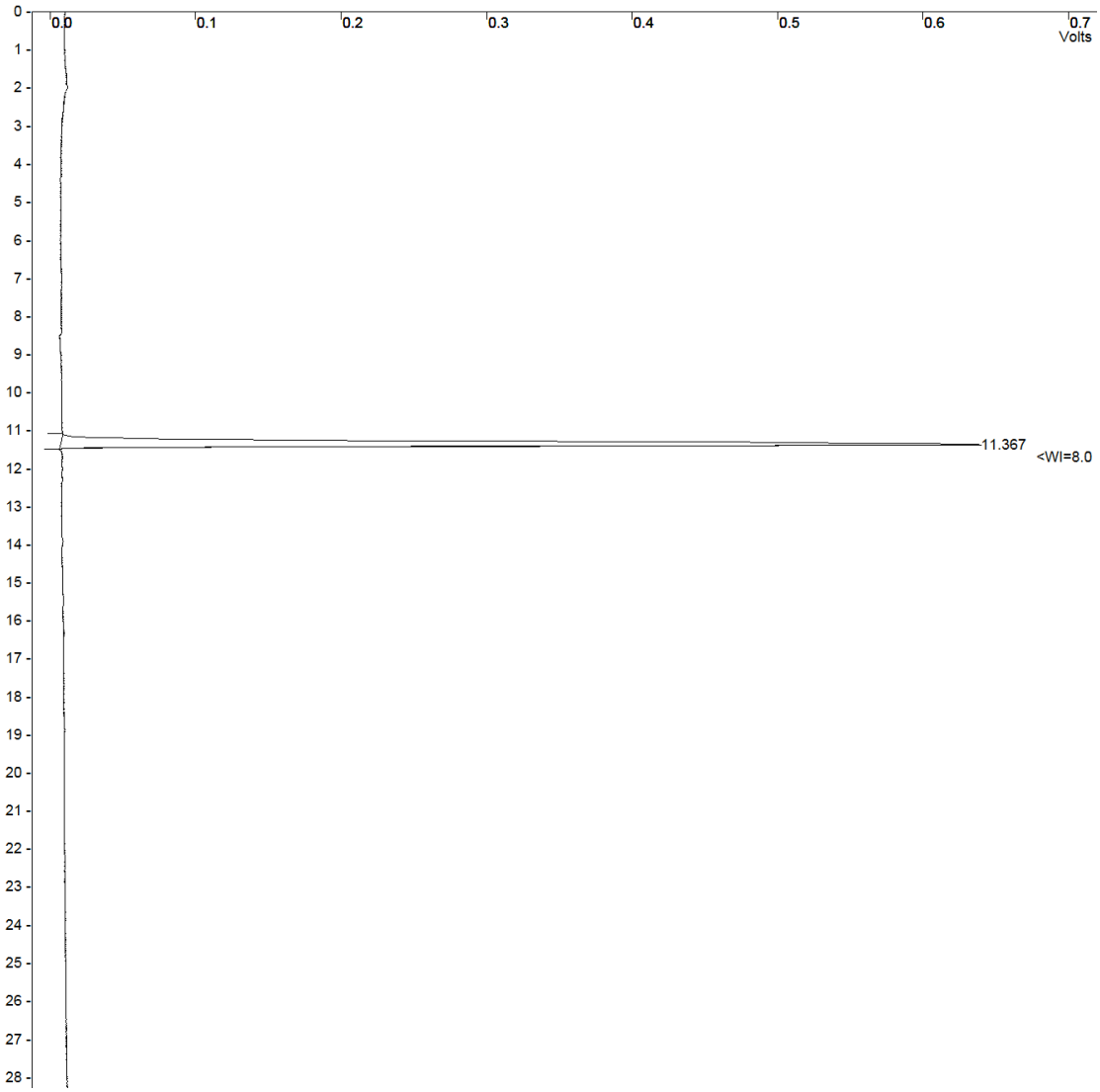
Compound Purity

Compound purity was determined by HPLC.

HPLC conditions:

1. Alltech C18 5μ 250mm x 4.6mm, 100% water (0-2 min), 0-100% acetonitrile over 23 min, 100% acetonitrile 3 min (25-28 min), 1mL/min, 50 μL injection. Detection was at 260 nm, run time 28 min.

```
Title       : fan-III-36 (ARB 05-100872)
```



Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Stat Code
1		100.0000	11.367	0.000	5062383	BB	7.7	
	Totals:	100.0000		0.000	5062383			