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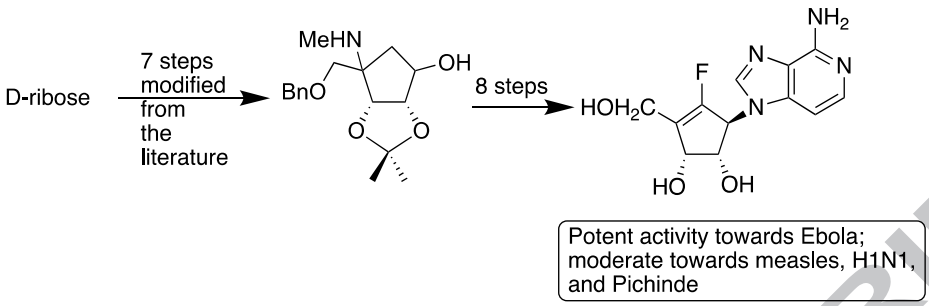
Graphical Abstract



Potent activity towards Ebola; moderate towards measles, H1N1, and Pichinde

6'-Fluoro-3-deazaneplanocin: synthesis and antiviral properties, including Ebola

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Abstract.

A convenient stereospecific synthesis of 6'-fluoro-3-deazaneplanocin (**6**) has been accomplished from D-ribose in 15 steps. It is reported to possess significant activity towards Ebola (Zaire, Vero, μM: EC_{50} < 0.36; CC_{50} 125; SI >347) with moderate inhibition of the target enzyme (S-adenosylhomocysteine hydrolase), which did not correlate directly with its anti-Ebola effects. Compound **6**, with limited cytotoxicity, also displayed activity against measles, H1N1 and Pichinde.

Keywords: carbocyclic nucleosides, 3-deazaneplanocin, Ebola, measles, H1N1, Pichinde virus, cytomegalovirus.

S-Adenosylmethionine (SAM) is the methyl donor cofactor for numerous biological methylations.[1] As a consequence of this process, SAM is converted to S-adenosylhomocysteine (SAH) that, subsequently, acts as a biofeedback inhibitor of the SAM transformations, and, thus, moderating further methylations.[2] S-Adenosyl-

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homocysteine hydrolase (SAHase) serves to remove the product SAH permitting further SAM methylations to occur.[2] Thus, SAHase is a significant control agent in this process and a site to target for affecting biomethylations.[2]

In this direction, adenine-based carbocyclic nucleosides based on aristeromycin (**1**) and neplanocin (**2**) have arisen as a source of SAHase inhibitors to control requisite methylations for antiviral drug discovery.[3] This has provided the impetus for our ongoing efforts of varying the known SAHase inhibitors 3-deazaaristeromycin (**3**) and 3-deazaneplanocin (**4**) to achieve agents with non-cytotoxic antiviral activity.[4] In the evolution of our program, we were drawn to a publication from Jeong's laboratory that, through extensive docking with human SAHase, reported 6'-fluoroneplanocin (**5**)[5] to have a dual mechanistic means of inhibition of SAHase. This prompted us to seek the 3-deaza analog (**6**) as a mechanistically-diverse target. This is described here.

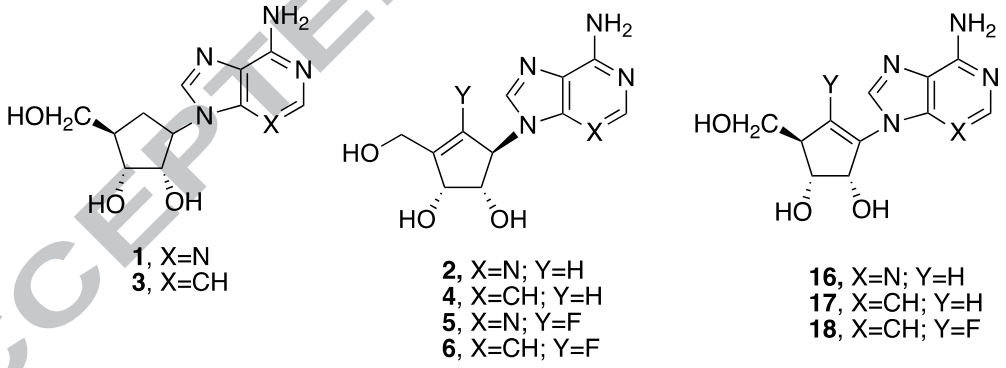
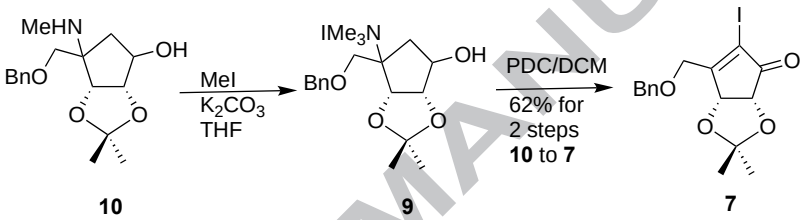


Figure 1. Relevant carbocyclic nucleosides

For this objective, our retro-synthetic thinking was built around cyclopentenyl iodide **7** (Scheme 1) whose corresponding alcohol (**8**, Scheme 2) could, in a protected form, participate in an electrophilic vinyl fluorination with a subsequent coupling reaction employing a protected 3-deazaadenine.

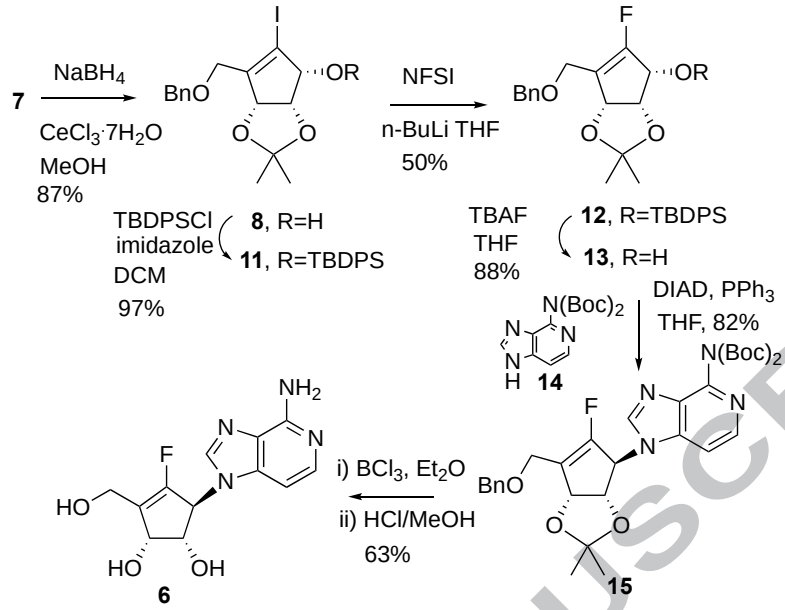
Few methods[6] exist for preparing α-iodo-α,β-unsaturated ketones (such as **7**). For our purpose, the little known oxidative cleavage of alcohols possessing a quaternary ammonium iodide at their 3-position drew our attention.[7] To consider this approach for obtaining **7** required accessing **9** from **10**, which was obtained from D-ribose by modifying a reported procedure[7] where the benzyl group replaced a trityl to improve the subsequent electrophilic fluorination reaction.[8] Oxidation of **9** with pyridinium chlorochromate yielded **7** via oxidative elimination, iodination of the resultant α,β-ketone and then elimination of hydrogen iodide (Scheme 1).



10 **9** **7**

Scheme 1. Synthesis of the requisite vinyl iodide **7.**

With **7** available, it was reduced under Luche conditions to alcohol **8** (Scheme 2) that was protected as the t-butyldiphenylsilyl derivative **11**. Fluorination with N-fluorobenzenesulfonimide (NFSI) gave **12.** Removal of the hydroxyl silyl group (to **13**)[9] followed by a Mitsunobu coupling with the N^6,N^6-protected 3-deazaadenine (**14**)[10] produced **15**. Boron trichloride followed by methanolic hydrochloric acid yielded the sought **6**.[11]



Scheme 2. Synthesis of the target compound **6.**

With **6** in hand, it was subjected to antiviral analysis.[12] A most pronounced activity was found versus Ebola (Zaire) as shown in Table 1with data for **2** and **4** and the 1',6'-isoneplanocins **16** and **17**, benchmark compounds of our Ebola program. From that data it can be seen that the anti-Ebola activity of **6** is comparable to **16** and **17** with an SI in line with **17**. The only reported antiviral data for **5** is versus vesicular stomatitis virus (VSV) (EC_{50} = 0.43 μM; CC_{50} = 40 μM). Since VSV and Ebola are (-)-RNA viruses and VSV has been used as a pseudotype Ebola virus in gene product studies[13a] and vaccine development,[13b] it is tempting to extrapolate the VSV results for **5** to its potential towards Ebola and conclude that **6** is more promising than **5** as an anti-Ebola candidate.

Table 1. *In vitro* activities against Ebola virus (Zaire) (μM)

Compounds	**6**	**2**[14]	**4**[14]	**16**[12b]	**17**[4]
Cell lines	Vero	Vero	Vero	Vero	Vero
EC_{50} (μM)	<0.36	4	2	0.38	<0.32
CC_{50} (μM)	125	60	>1700	1.3	>100
SI	>347	15	>850	3.5	>313

In the other viral evaluations, **6** displayed activity[12] versus (i) measles (Vero 76) (EC_{50} < 0.36 μM; CC_{50} 115μM; SI >320); (ii) influenza A H_1N_1 (MDCK) (EC_{50} 20.2 μM; CC_{50} 133 μM; SI 6.6); and (iii) Pichinde (Vero) (EC_{50} 57.6 μM; CC_{50} 360 μM; SI > 6.3).

To determine if the antiviral effects of **6** could be an outcome of its inhibition of SAHase,[12h] this was investigated (Table 2). Its enzymatic IC_{50} was several fold less than **2**, **4**, **16**[12b] and **17**[4] (the latter two are lead compounds in our isoneplanocin Ebola studies, Figure 1) but, surprisingly, 50 times more potent than **5**. This latter observation is problematic and requires further analysis of the reported data[5] for **5**. With the weaker effect of **6** than expected based on **2**, **4**, **16** and **17** on SAHase but yet with its potent anti-Ebola activity, it could be that **6** is acting by more than simply SAHase inhibition.

Table 2. Inhibitory results against SAHase						
Compounds	**6**	**2**[12b]	**4**[12b]	**16**[12b]	**17**[4]	**5**[5]
IC_{50} (nM)	8.69	0.9	2.0	0.9	3.2	480

Compound **6** was inactive towards: Chikungunya (Vero 76), dengue virus 2 (Vero 76), polio virus 1 (Vero 76), Punta Toro (vero 76), Rift Valley fever (Vero 76), Tacaribe (Vero), Venezuelan equine encepthalitis (Vero 76), West Nile (Vero 76), Yellow fever (Vero), norovirus (HG 23), human cytomegalovirus (HFF); hepatitis B virus (2.2.15), adenovirus 5(A549), Utusu (vero 76), and Japanese equine encephalitis (Vero 76).

In conclusion, by considering the antiviral properties of 3-deazaneplanocin (**4**) and 6'-fluoroneplanocin (**5**) and combining their structural features, 6'-fluoro-3-deazaneplanocin (**6**) arose as a target for our antiviral program. Thus, an efficient and practical synthesis of **6** is reported in 15 steps from D-ribose. While the antiviral effects of **6** were not as broad as desired, its potential versus Ebola, measles, H1N1 and Pichinde (all RNA viruses) suggest **6** as a prototype compound for antiviral agent design (for example, by combining the features of **6** and **17** as represented by **18**). This is being evaluated in our laboratory at Auburn together with designing experiments to clarify the mechanism of antiviral action of **6** beyond SAHase, also, for analog development.

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11. Compound **6**: white solid, ^{1}H NMR (600 MHz, CD_3OD) δ 8.16 (s, 1 H), 7.73 (d, *J* = 6.0 Hz, 1H), 6.93 (d, *J* = 6.0 H, 1H), 5.54 (s, 1H), 4.80 (t, *J* = 4.8 Hz, 1H), 4.49 (d, *J* = 13.2 Hz, 1H), 4.37 (t, *J* = 6.0 Hz, 1H), 4.22 (m, 1H); ^{13}C NMR (150.9 MHz, CD_3OD) 152.8 (*J* = 286.7 Hz), 152.1, 141.3, 139.9, 138.4, 126.8, 122.0, 97.5, 74.5 (*J* =6.0 Hz), 69.3 (*J* = 9.1 Hz), 63.2 (*J* = 18.1 Hz), 52.8; HRMS (ESI) calculated for $C_{12}H_{14}N_4O_3F$: 281.1050, Found $(M+H)^+$ 281.1048.

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