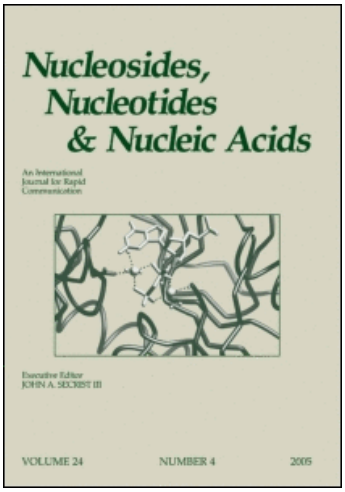


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# Synthesis and Biological Evaluation of Halo-neplanocin A as Novel Mechanism-Based Inhibitors of *S*-Adenosylhomocysteine Hydrolase

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## ABSTRACT

Halogenated analogues of neplanocin A were synthesized from the key intermediate **1**, among which fluoro-neplanocin A was found to be novel mechanism-based irreversible inhibitor of *S*-Adenosylhomocysteine hydrolase.

*Key Words:* Halo-neplanocin A; *S*-Adenosylhomocysteine hydrolase; Mechanism-based inhibitor.

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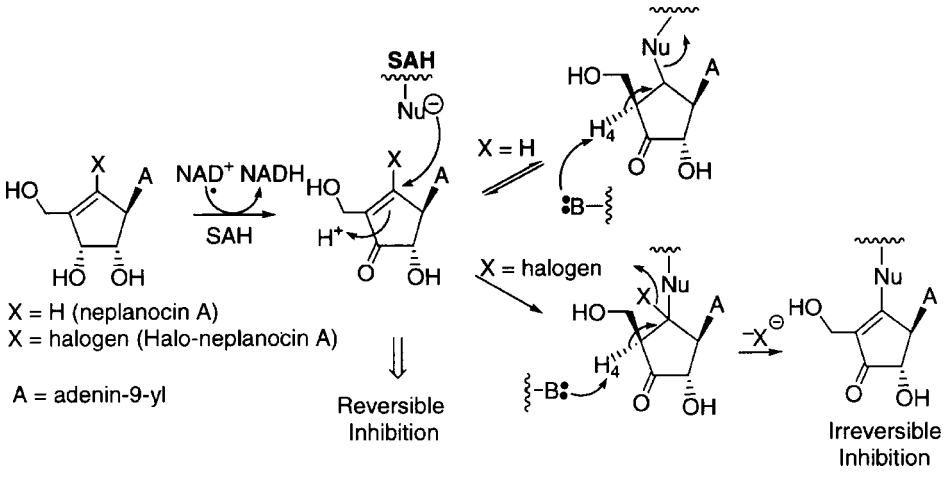
*S*-Adenosylhomocysteine hydrolase (SAH)[1,2] catalyzes the hydrolysis of *S*-adenosylhomocysteine to adenosine and L-homocysteine. Inhibition of this enzyme accumulates *S*-adenosylhomocysteine, which in turn inhibits *S*-adenosyl-L-methionine dependent transmethylation, resulting in no formation of the capped methylated structure at the 5′-terminus of viral *m*RNA.[1,2] Thus, SAH has been an attractive target for the development of broad spectrum of antiviral agents.[3]

Neplanocin A has been recognized as one of the most potent inhibitors of SAH by depleting enzyme-bound cofactor $NAD^+$.[4–6] This inhibition is reversed by the addition of cofactor $NAD^+$. In addition to this well-known cofactor depletion mechanism, it is mechanistically hypothesized that neplanocin A may form a covalent bond with a nucleophilic amino acid residue at the active site of the enzyme through a Michael type reaction, but its irreversible action may be easily reversed by the presence of acidic 4′-hydrogen as shown in Sch. 1. Therefore, based on this reversible addition-elimination hypothesis, we wanted to demonstrate the likelihood of this mechanism by designing halo-neplanocin A analogues which may be able to inhibit SAH irreversibly because of no acidic hydrogen at the 4′-position, as illustrated in Sch. 1.

Synthesis of the halo-neplanocin A analogues started from the known key intermediate **1**,[7] as shown in Sch. 2.

The intermediate **1** was treated with chlorine, bromine or iodine in the presence of pyridine to give the halogenated ketones **2**. Reduction of **2** with sodium borohydride in the presence of cerium (III) chloride gave the allylic alcohols **3**. After mesylation of **3**, the mesylates **4** were condensed with adenine anion in the presence of 18-Crown-6 to yield the protected nucleosides. Treatment of the protected nucleosides with boron trichloride at −78°C afforded the final nucleosides **5**. For the synthesis of the fluoro-neplanocin A (**8**), iodo derivative **3** was protected as *t*-butyldiphenylsilyl ether **6**. Reaction of **6** with *N*-fluorobenzenesulfonimide[8] followed by deprotection with *n*-tetrabutylammonium fluoride produced the desired vinylfluoride **7**




***Scheme 1.*** Proposed mechanism for the reversible (neplanocin A, X = H) and irreversible (fluoro-neplanocin A, X = halogen) reactions at the active site of SAH.





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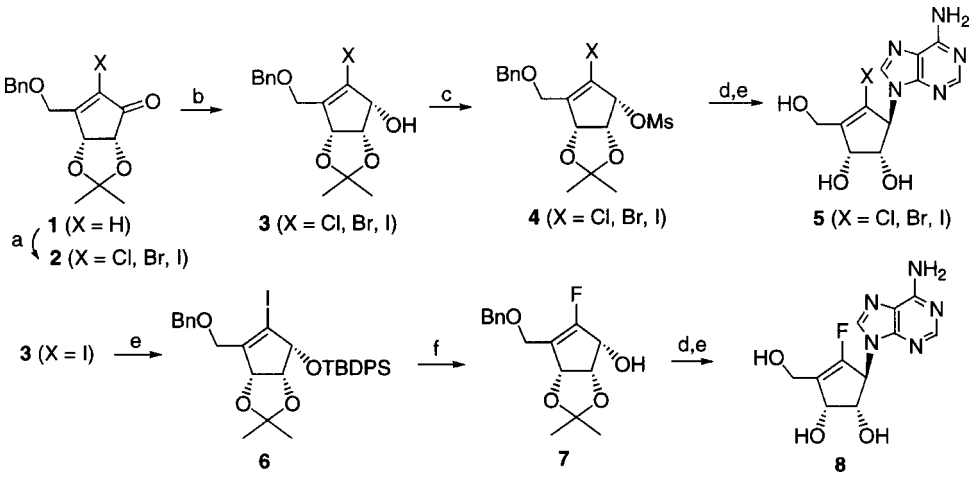


***Scheme 2.*** Reagents: a) $X_2$, $CCl_4$, pyridine, 53–70%; b) $NaBH_4$, $CeCl_3$-$7H_2O$, 85–97%; c) MsCl, $El_3N$, 89–99%; d) adenine, 18-Crown-6, DMF, 40–73%; e) 1 N $BCl_3$, $CH_2Cl_2$, 50–70%; e) TBDPSCl, DMF, 50°C, 97%; f) *n*-BuLi, *N*-fluorobenzenesulfonimide, −78°C, then *n*-$Bu_4NF$, THF, 63%.

in 63% yield. According to the similar procedure used in the preparation of **5**, vinyl-fluoride **7** was transformed to the fluoro-neplanocin A (**8**).

Inhibition of SAH by neplanocin A and its halogenated analogues, **5** and **8** was measured using pure recombinant enzyme from human placenta. The results showed that compound **8** ($IC_{50} = 0.48\,\mu M$) was *ca.* 2-fold more potent than the parent neplanocin A ($IC_{50} = 0.87\,\mu M$). However, the chloro ($IC_{50} = 36.46\,\mu M$) and bromo ($IC_{50} = 60.17\,\mu M$) derivatives were found to be less potent than neplanocin A and iodo derivative ($IC_{50} = 1\,mM$) was inactive. It is interesting to note that enzyme inhibitory activity is inversely proportional to the size of the halogen atom, indicating the binding pocket of the halogen atom is very small. The irreversible nature of the inhibition achieved with **8** was demonstrated using dialysis, incubation with $NAD^+$ or adenosine, and $^{19}F$ NMR experiment, indicating that fluoro-neplanocin A (**8**) is the mechanism-based inhibitor of SAH that appears to operate by our proposed mechanism.

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