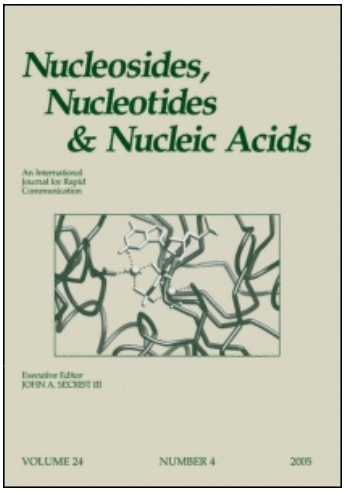

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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF SUGAR-MODIFIED 2-(*p*-*n*-BUTYLANILINO)-2'-DEOXYADENOSINE ANALOGUES

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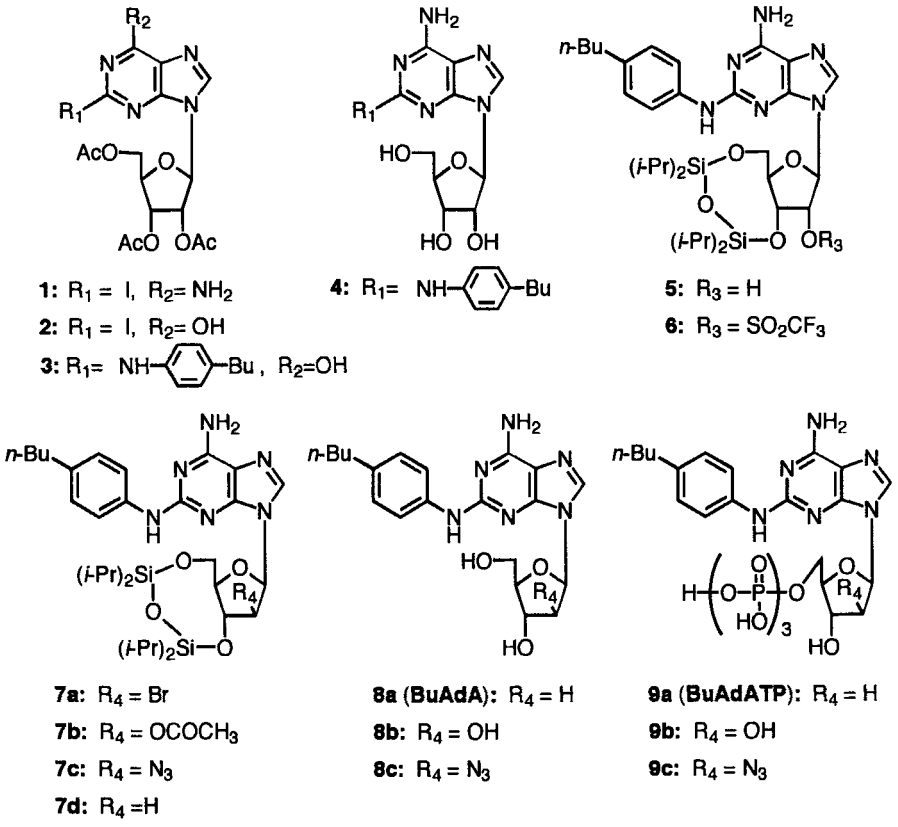
Abstract: Several sugar-modified 2-(*p*-*n*-butylanilino)-2'-deoxyadenosine analogues, including arabino and 2'(*R*)-azido-2'-deoxy analogues and their 5'-triphosphates were synthesized. These nucleosides thus obtained exhibited moderate cytotoxicity against P-388 leukemic cells in culture (IC_{50} = 13–24 μM). In contrast to above results, the 5'-triphosphates have been shown to exert strong and selective inhibitory effects on mammalian DNA polymerase α (*Ki*=0.02–0.04 μM).

It has been reported that 5'-triphosphates of 2-(*p*-*n*-butylanilino)-2'-deoxyadenosine (BuAdA, **8a**) and 2-(*p*-*n*-butylphenyl)-2'-deoxyguanosine (BuPdG) are potent and selective inhibitors of eukaryotic DNA polymerase α.[1] These compounds inhibit DNA polymerase α with *Ki* values in the nanomolar range by competing with the natural substrate dATP or dGTP. It has also been reported that BuAdA and BuPdG exhibit moderate cytotoxic activity *in vitro*, however, these compounds have not shown activity against P-388 leukemia in mice.[2] It is expected that 2'(*R*)-substituted derivatives of nucleosides may exhibit more potent biological activities than the original compounds. Therefore, the synthesis of the sugar-modified BuAdA analogues was considered.

For the synthesis of purine nucleosides bearing *p*-*n*-butylanilino group at C-2, triacetyl-2-iodoadenosine[3] (**1**), which is readily prepared from guanosine, was used as the starting material. However, it is known to be difficult to replace the 2-halogeno group of 2-halogenoadenosine with aniline.[4] Thus, triacetyl-2-iodoadenosine (**1**) was first converted to triacetyl-2-iodoinosine (**2**) by deamination reaction in 84% yield. Heating of triacetyl-2-iodoinosine with *p*-*n*-butylaniline in methanol afforded triacetyl-2-(*p*-*n*-



butylanilino)inosine (**3**) in 75% yield. The chlorination of **3** followed by reaction with methanolic ammonia gave 2-(*p*-*n*-butylanilino)adenosine (**4**). The 2'-modified nucleosides were synthesized essentially by the method of Fukukawa et al.[5] The 3'- and 5'-hydroxyls of **4** were protected with tetraisopropyldisiloxane-1,3-diyl group and converted to the 2'-*O*-triflate (**6**). Nucleophilic displacement of the leaving group of **6** by Br^-, AcO^- and N_3^- afforded the respective 2'(*R*)-substituted products **7a**, **7b** and **7c**. Reduction of **7a** with tri-*n*-butyltin hydride and azobis(isobutyronitrile) in toluene at reflux temperature yielded the 2'-deoxy derivative **7d**. Deprotection of **7d**, **7b** and **7c** afforded **8a** (BuAdA), **8b** (BuAaraA) and **8c** (2'-azidoBuAdA), respectively.

In order to examine the inhibitory effects on the DNA polymerases, nucleosides **8b** and **8c** were converted into the corresponding 5'-monophosphate derivatives by phosphorylation with $POCl_3$ in triethyl phosphate, and then the nucleotides were further converted to their 5'-triphosphates **9b** (BuAaraATP) and **9c** (2'-azidoBuAaraATP) using the phosphoroimidazolidate method.

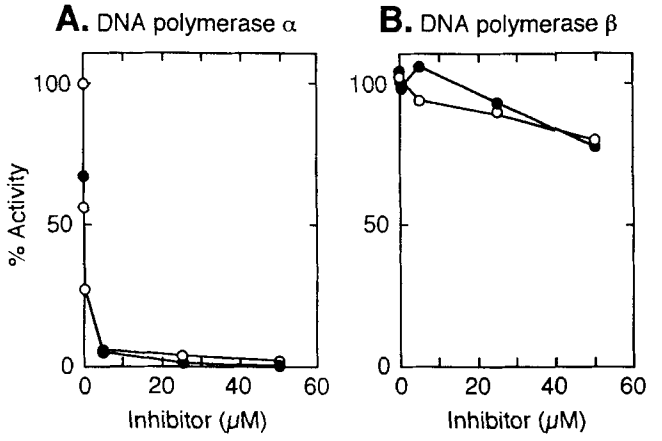


Figure 1. Inhibitory effects of BuAaraATP (**9b**) (-○-) and 2'-azido BuAaraATP (**9c**) (-●-) on eukaryotic DNA polymerase α (panel **A**) and β (panel **B**).

Reactions were carried out for 20 min at 37 °C with activated calf thymus DNA as the template-primer in the presence of 50 μM [^{3}H]dATP

The compounds **8a**, **8b** and **8c** showed growth inhibitory action against P-388 leukemic cells in culture with IC_{50} values of 16.3, 24.2 and 13.6 μM, respectively. Activity of these analogues against HSV-1 was examined and *in vitro* and the compounds were found to be essentially inactive up to 25 μg/ml.

We examined the inhibitory effects of BuAaraATP (**9b**) and 2'-azidoBuAaraATP (**9c**) on calf thymus DNA polymerase α and rat DNA polymerase β with activated calf thymus DNA as the template-primer. As shown in Figure 1, DNA polymerase α was inhibited strongly by both analogues. From the double reciprocal plots the modes of inhibition by these analogues were competitive with respect to dATP. The *Ki* values of BuAaraATP (**9b**) and 2'-azidoBuAaraATP (**9c**) were determined to be 0.017 and 0.038 μM, respectively. The inhibitory effect of BuAaraATP (**9b**) was comparable to that of BuAdATP (**9a**) (*Ki* = 0.008 μM [6]). In contrast, DNA polymerase β was not or only slightly inhibited by these dATP analogues bearing *p-n*-butylanilino group at the 2-position. Although the 5'-triphosphate derivatives of BuAaraA (**8b**) and 2'-azidoBuAaraA (**8c**) were shown in the present study to be the potent and selective inhibitors of DNA polymerase α, these nucleosides (**8b** and **8c**) did not exhibit significant cytotoxicity against murine leukemic cells in culture. Probably these compounds are poor substrates for cellular kinases.

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