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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 (IC₅₀ = 13-24 μ M). 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 μ M).

It has been reported that 5'-triphosphates of 2-(p-n-butylanilino)-2'-deoxyadenosine (BuAdA,**8a**) and <math>2-(p-n-butylphenyl)-2'-deoxyguanosine (BuPdG) are $potent and selective inhibitors of eukaryotic DNA polymerase <math>\alpha$.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*-butylaniline).

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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 N₃⁻ 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 POCl₃ 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) (-o-) and 2'-azido BuAaraATP (9c) (-o-) 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 [³H]dATP

The compounds **8a**, **8b** and **8c** showed growth inhibitory action against P-388 leukemic cells in culture with IC₅₀ values of 16.3, 24.2 and 13.6 μ M, 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 μ g/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 μ M, respectively. The inhibitory effect of BuAaraATP (9b) was comparable to that of BuAdATP(9a) (*Ki* = 0.008 μ M⁶). In contrast, DNA polymerase β was not or only slightly inhibited by these dATP analogues bearing *p-n*-butylanilino group at the 2position. 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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