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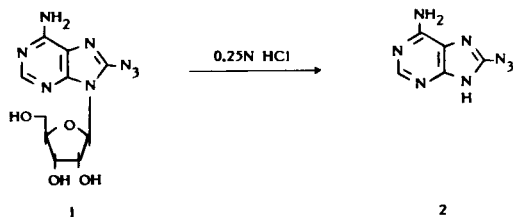
The photoaffinity labeling agent, 8-azidoadenine, was prepared in good yield by hydrolytic cleavage of 8-azidoadenosine and characterized as a homogeneous compound. Previously reported physical data for this compound was shown to be in accord with mixtures of 8-azido- and 8-bromoadenine.

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The use of 8-azidopurine nucleosides and nucleotides as photoaffinity labeling reagents is common (1) but the parent purine 8-azidoadenine (2) has only recently been reported (2). We had previously synthesized 8-azidoadenine by a different method and found that the spectral properties of our material were different from those reported. In our hands a repetition of the literature procedure for the synthesis of 2 by nucleophilic substitution at C-8 of purine afforded a product that is roughly a 1:1 mixture of the starting material, 8-bromoadenine (3) and 2. Even under more forcing conditions the reaction did not go to completion.

Since there is potential utility of 2 as a photoaffinity labeling reagent and since under certain conditions 8-bromoadenine is not photostable (*i.e.*, irradiation with a 450w Hanovia lamp in quartz), we describe here our procedure for the preparation of 2 and give evidence for our assertion that the published product is a mixture.

Pure 8-azidoadenine (2) has been synthesized by hydrolytic cleavage of the ribose moiety from the readily accessible (3) 8-azidoadenosine (1):



The product 2 has been characterized by elemental analysis, ms, nmr, ir and uv spectroscopy. The observed uv maximum for 2 was in accord with a prediction (4) of the  $\lambda$  max of the starting nucleoside 1 (See Table 1). The published spectrum of 2 differed from our observed spectrum and the predicted  $\lambda$  max. Repetition of the published procedure (2) afforded material whose elemental bromine analysis indicates that about 50% of the material is the starting compound, 8-bromoadenine (3). Qualitatively this is confirmed by the mass spectrum (Fig. 1) which shows an intense molecular ion doublet at  $m/e$  213 and 215. A 1:1 mixture of pure azidoadenine and 3 affords a uv spectrum (Fig. 2) whose shape and  $\lambda$  max is the same as that obtained from the material prepared according to (2). In addition, the PMR spectrum shows an absorption of equal in-

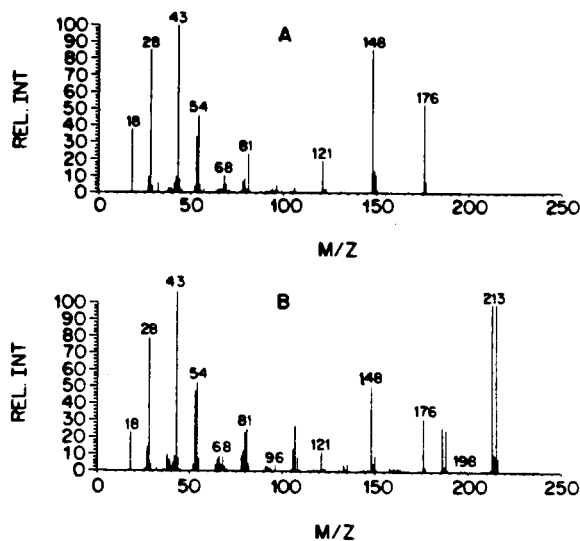


Figure 1: Mass Spectra of 8-azidoadenine Preparations. A: Prepared by hydrolysis of 8-azidoadenosine. B: Prepared according to procedure in reference 2.

tensity in the 8 ppm region (*i.e.*, C-2 H). Experimentally, the most diagnostic indication of 8-bromoadenine contamination of the reaction product is the mass spectrum shown in Fig. 1. The presence of a linear azide and its substitution in a five-membered ring (5) is supported by the ir spectrum.

The use of 2 as a photoaffinity reagent will be limited by its relative insolubility at pH 7.4 (*ca.* 0.8 mM). However, 2 prepared by our procedure should avoid any complications afforded by the presence of 3.

TABLE I  
Ultraviolet Spectra of Various Adenine Analogs<sup>a</sup>

Compound	$\lambda_{\max}$	$\epsilon \times 10^{-4}$
8-Azidoadenine (2)	284	2.10
8-Bromoadenine	266	1.48
8-Azidoadenosine	281	1.91
	282 <sup>b</sup>	1.45
8-Azidoadenine <sup>c</sup>	< 280	
8-Azidoadenine <sup>d</sup>	274	

<sup>a</sup>All spectra obtained in 0.25N HCl except where noted otherwise. <sup>b</sup>In pH 7.4 phosphate buffer. <sup>c</sup>Published in ref 2. <sup>d</sup>Prepared according to ref 2.

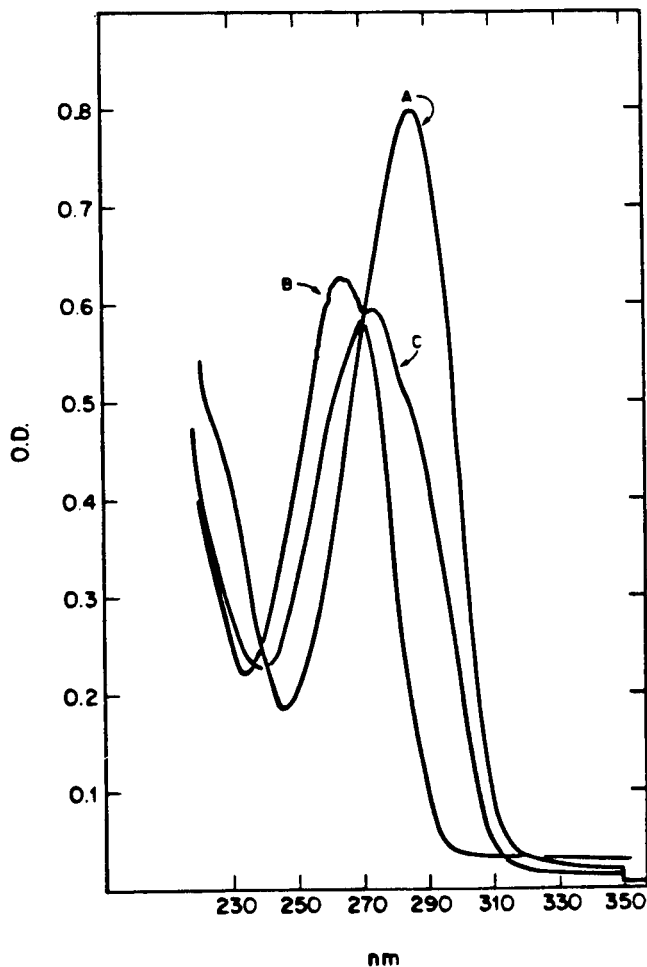


Figure 2: Ultraviolet Spectra in 0.25M Hydrochloric Acid. A:  $3.8 \times 10^{-5} M$  8-azidoadenine (**2**). B:  $1.86 \times 10^{-5} M$  8-bromoadenine (**3**). C:  $1.86 \times 10^{-5} M$  **3** plus  $1.91 \times 10^{-5} M$  **2**.

#### EXPERIMENTAL

##### 8-Azidoadenine (**2**).

8-Azidoadenosine (**3**) (I, 308 mg, 1 mmole) was dissolved in 0.25N hydrochloric acid (65 ml) and the solution was protected from light and heated at reflux for 5 hours. After concentration to dryness *in vacuo*, the residue was dissolved in water (10 ml) and made neutral (pH 6.85) with 10 ml of 5% aqueous sodium bicarbonate. The resulting suspension was aged for 3 days at 4°, filtered and the solid washed with  $3 \times 5$  ml of water, 150 mg (85%) of 8-azidoadenine (**2**) was obtained (mp, explodes at 185°

when inserted at 180°). A sample dried for analysis at 80° in high vacuum did not lose more than 1% weight; 200 MHz nmr (DMSO- $d_6$ ): 7.2  $\delta$  (3H, broad s, disappears on exchange with perdeuteriomethanol), 8.1 (1H, sharp singlet); ir (nujol mull): 2.92, 3.05  $\mu$  ( $NH_2$ ) 3.20 (N-H) 4.66 ( $N_3$ ). (0.25N hydrochloric acid); See Table 1 and Figure 2. ms: see Figure 1; high resolution ms: Calcd. for  $C_5H_4N_8$ : 176.05589. Found: 176.05591.

Anal. Calcd. for  $C_5H_4N_8$ : C, 34.09; H, 2.27; N, 63.63. Found: C, 33.85; H, 2.13; N, 62.86.

A *p*-toluenesulfonic acid salt was prepared by admixture of **2** and an equivalent quantity of *p*-toluenesulfonic acid followed by trituration to afford the salt, mp 197-198° dec.

Anal. Calcd. for  $C_{12}H_{12}N_8SO_3$ : C, 41.38; H, 3.45; N, 32.18; S, 9.20. Found: C, 41.21; H, 3.46; N, 31.98; S, 9.22.

"8-Azidoadenine" Prepared According to the Procedure in Reference (2).

8-Bromoadenine (300 mg, 1.4 mmoles, Sigma) and sodium azide (204 mg, 3.1 mmoles) were dissolved in *N,N*-dimethylformamide (DMF) (50 ml) and the solution heated at 80° in the dark for 15 hours. The DMF was removed *in vacuo* at 40° and the residue dissolved in 0.5M aqueous ammonia (4 ml). This solution was acidified to pH 4.8 with 2.5N hydrochloric acid (0.7 ml), at which point a copious precipitate appeared. It was filtered and dried to give 346 mg of a tan powder.

Anal. Calcd. for  $C_5H_4N_8$ : C, 34.09; H, 2.27; N, 63.63. Found: C, 26.98; H, 2.06; N, 40.97; Br (total), 15.12; Br (ionic), 1.38.

One hundred mg of this material was shaken in a pH 2 phosphate buffer (1 ml) for 1 hour and filtration afforded 46 mg of product. Found: C, 22.89; H, 2.31; N, 34.40; Br, 14.79; 200 MHz, nmr (DMSO- $d_6$ ): 2 lines at 8.05  $\delta$  of roughly equal intensity, 2 active hydrogen atoms at 7.2 and 7.4. The uv spectra are shown in Table 1 and Figure 2. The mass spectrum is shown in Figure 1. A similar procedure was applied to a different sample except that the aqueous ammonia solution was adjusted directly to pH 1.9 with 2.5N hydrochloric acid. The product obtained was the same as the one formed by the pH 2 slurry of the material isolated at pH 4.8.

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