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THE SYNTHESIS OF 2-AZA-ADENOSINE-3',5'-CYCLIC PHOSPHATE *VIA* 1,N^6-ETHENO-ADENOSINE-3',5'-CYCLIC PHOSPHATE

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Treatment of 2-aza-1,N^6-etheno-c-AMP with NBS or Br_2 under mild conditions gave 2-aza-c-AMP in a high yield. Therefore, the route c-AMP ⟶ 2-aza-1,N^6-etheno-c-AMP ⟶ 2-aza-c-AMP offers an excellent way to synthesize the last compound.

Synthesis of 2-aza-c-AMP from c-AMP has been reported[1]. But the yield was not high, because the procedure involved multiple steps and unstable intermediates. A convenient synthesis of 2-aza-c-AMP is very important in order to obtain further information concerning the unique properties of 2-aza-c-AMP analogues in the biological system[2]. We now find a new synthetic method of 2-aza-c-AMP (V) from 1,N^6-etheno-c-AMP (II). The synthesis of II was described in literatures[3,4] and the compounds (III and IV) were synthesized by the method of Yip and Tsou[5], but the data of NMR and the solvent for crystallization were not reported. We now report the properties of III and IV and the conversion of the latter (IV) to the titled compound (V). This method is superior to the reported method[1] for its simplicity and high yield.

The compound (II) (4.0 g) was treated with 1 N sodium hydroxide (40 ml) at room temperature overnight. The reaction mixture was concentrated and acidified (pH 2) to obtain crystalline 3-β-D-(3',5'-cyclic phospho)-ribofuranosyl-4-amino-5-(imidazol-2-yl)-imidazole (III) (yield 80%). The NMR spectrum in D_2O showed imidazole protons as two singlets at δ 7.65 (2H) and δ 8.68 (1H). The compound (III) (4.0 g) was reacted with sodium nitrite (0.84 g) in 80% acetic acid (240 ml) at room temperature for 30 min. The reaction mixture was then concentrated and acidified (pH 2) to obtain crystalline 2-aza-1,N^6-etheno-c-AMP (IV) (yield 94%). The NMR spectrum in D_2O showed etheno protons as a sets of doublets centered at δ 7.22 and δ 7.79 and C_8-H as a singlet at δ 8.24.

The compound (IV) (1.0 g) was stirred overnight in DMF-H_2O (1:1) (200 ml) in the presence of N-bromosuccinimide (NBS) (6.0 g). By this treatment, fluorescence due to the etheno group was lost. The reaction mixture was passed through a column of alumina, washed with water and eluted with NH_4OH (28% aq.)-H_2O-EtOH (1:5:5) (200 ml). The eluate was concentrated and again applied to a Dowex-50 (H^+) column and the eluates (eluting solvent, H_2O) were collected. The fraction containing uv-absorbing material (300 ml) was evaporated in vacuo. Recrystallization of the residue from water gave colorless crystals (yield 80%). The compound (V) was characterized by elemental analysis ($C_9H_{11}N_6O_6P \cdot 2H_2O$ requires C, 29.52; H, 4.13; N, 22.95%. Found: C, 29.55; H, 4.06 and N, 22.12%), ultraviolet absorption spectra[1], and NMR spectrum in D_2O [the one aromatic proton signal (C_8-H) appeared as a singlet at δ 8.31, and the ribose proton signals were almost the same as those of c-AMP[6] (C_1' proton at δ 6.10, C_2'-C_5' protons at δ 4.20-4.70)]. The 2-aza-c-AMP we prepared can be re-converted to the etheno compound (IV) by treatment with $ClCH_2CHO$.

The yield of the product (V) from the etheno compound (IV) depends on the amount of H_2O in DMF, and on the pH of the solution when the reaction is performed in acetate buffer (Table 1). In the H_2O/DMF system, water not only

Table 1. Yield of 2-aza-c-AMP from the etheno compound (IV) under various conditions*.

	Percentage of H_2O in DMF					pH of 1 M acetate buffer				
	0	30	50	70	100	2.5	3.0	4.0	5.0	6.0
Yield(%) of 2-aza-c-AMP**	63	100	100	93	65	75	84	83	83	65

*Reaction conditions: 2-aza-1,N^6-etheno-c-AMP, 10 mg; NBS, 100 mg; solvent, 10 ml; and reaction time, 18 hr.

**The yield of 2-aza-c-AMP was determined by paper chromatographic separation, followed by the determination of the absorbance. Solvent (n-Butanol:Acetic acid: Water=5:2:3), Rf=0.31.

enhances the solubility of IV but also plays a significant role in determining the yield of the product. However, in higher concentrations (≧70%), the formation of by-products was observed. In the acetate buffer system, this reaction has the optimum pH at about 4.0.

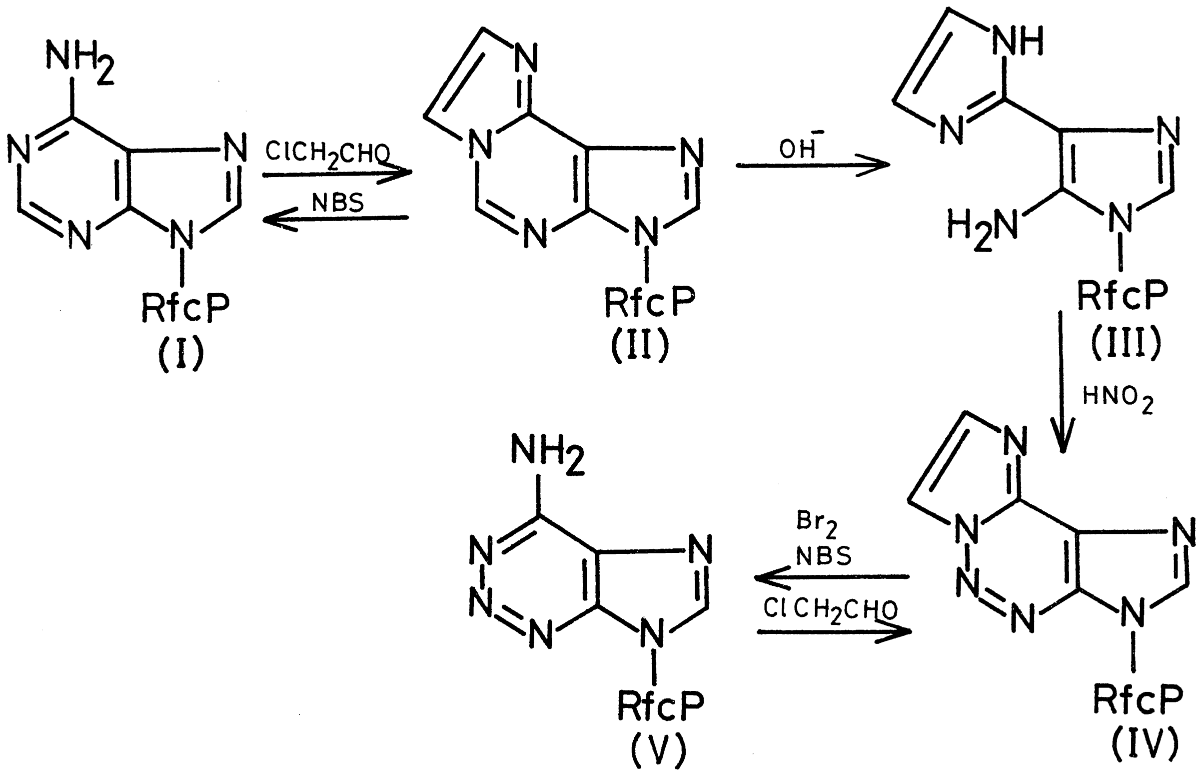
The deblocking of the etheno group can also be effected by Br_2 instead of NBS (Table 2). The compound (II) can be converted to c-AMP (I) by the treatment with NBS.

Table 2. Yield of 2-aza-c-AMP from the etheno compound (IV) with Br_2 in 1 M acetate buffer solution (pH 4.0)*.

Amount(mg) of Br_2	25	50	75	100	125
Yield(%) of 2-aza-c-AMP**	60	89	80	60	55

*Reaction conditions: 2-aza-1,N^6-etheno-c-AMP, 20 mg; solvent, 10 ml; and reaction time, 18 hr.

**The yield of 2-aza-c-AMP was determined by the procedure identical with that described in Table 1.



RfcP: β-D-(3',5'-cyclic phospho) ribofuranosyl

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