# SYNTHESIS OF 8-AZIDOADENOSINE 5'-PHOSPHATE*

Josef HOUŠTĚK[a] and Jiří SMRT[b]

[a] *Physiological Institute, Czechoslovak Academy of Sciences, 142 00 Prague 4 and*
[b] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*

Received May 3rd, 1978

Sodium azide reacts with 8-bromoadenosine 5'-phosphate (*I*) in dimethyl sulfoxide under the formation of 8-azidoadenosine 5'-phosphate (*II*). The compound *II* was prepared alternatively from 8-azidoadenosine (*V*) and phosphoryl chloride.

Photoaffinity labelling 8-azidoadenosine 5'-triphosphate has been prepared from 8-azidoadenosine 5'-phosphate[1-3] (*II*). For the synthesis of compound *II*, reaction of 8-bromoadenosine 5'-phosphate (*I*) with azides of tributylamine[1], trioctylamine[2] and tetramethylguanidine[3] were used. We describe now the use of sodium azide for this conversion, in analogy with the preparation of 8-azidoadenosine 3',5'--cyclic phosphate[4]. The reaction proceeds in dimethyl sulfoxide and gives, after 18 h at 80°C, 65% yield of phosphate *II*. The product is isolated by chromatography on Dowex-1 ($HCOO^-$) using gradient of formic acid. The isolated form (free acid) allows the direct use in Michelson's[5] procedure for triphosphate synthesis.

Alternative route to the synthesis of phosphate *II* would employ the phosphorylation of 8-azidoadenosine (*V*) or its derivatives. For this approach we prepared 2',3'-O-ethoxymethylene-8-bromoadenosine (*IIIa*) and 2',3'-O-isopropylidene-8-bromoadenosine (*IIIb*) from 8-bromoadenosine. The compounds *IIIa* and *IIIb* were converted to 8-azido derivatives *IVa*, *IVb* by the action of sodium azide in dimethyl sulfoxide. Both products were readily separated from inorganic salts and dimethyl sulfoxide by chloroform extraction. Simple isolation of *IVa* and easy removal of ethoxymethylene group allowed convenient preparation of 8-azidoadenosine which was then phosphorylated by phosphoryl chloride in triethyl phosphate according to Yoshikawa[6].

* Part CXCVIII in the series Nucleic Acids Components and their Derivatives; Part CXCVII: This Journal *44*, 613 (1979).

NaN3
(CH3)2SO
1. POCl3
2. (C2H5)3N
NaN3
H+
I
II
V
IIIa, R1 = H, R2 = OC2H5
IIIb, R1 = R2 = CH3
IVa, R1 = H, R2 = OC2H5
IVb, R1 = R2 = CH3
V

## EXPERIMENTAL

Thin-layer chromatography was performed on ready-for-use Silufol $UV_{254}$ silica gel foils (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent systems $S_1$, 2-propanol–conc. ammonia–water (7 : 1 : 2); $S_2$, chloroform–methanol (9 : 1); $S_3$, chloroform–methanol (85 : 15). Unless stated otherwise, solutions were taken down on a rotatory evaporator at 40°C, 15 Torr. All manipulations with 8-azidoadenosine containing compounds were carried out in flasks wrapped in aluminium foils. UV spectra were performed on apparatus Specord UV VIS (Carl Zeiss, Jena).

8-Bromoadenosine 5'-Phosphate (*I*) (Modification of published procedure[7])

Bromine (0·61 ml) is added to the stirred solution of adenosine 5'-phosphate (10 mmol) and lithium hydroxide (20 mmol) in water (100 ml). Stirring is continued for 3 h, the resulting solution is evaporated, the residue dissolved in water (50 ml) and applied to a column (3·5 × 13 cm). of Dowex-1 ($HCOO^-$). The column is eluted with water (150 ml) and then with the use of linear gradient (4 l of water, 4 l of 1M formic acid). Unreacted adenosine 5'-phosphate is eluted with 0·15M formic acid, the product with 0·57M formic acid. The collected fractions are evaporated and the residue coevaporated with three 20 ml portions of water. The residue is dissolved in water (15 ml), and ethanol (300 ml) and ether (300 ml) are added. The crystalline product is collected, washed with ether and dried under diminished pressure. Yield 2·5 g (60%).

8-Azidoadenosine 5'-Phosphate (*II*)

A): The mixture of 8-bromoadenosine 5'-phosphate (*I*; 2 mmol) and sodium azide (10 mmol) is dissolved by heating in dimethyl sulfoxide (10 ml), and the solution heated to 80°C for 18 h. Water (200 ml) is added and the solution applied to a column (3·5 × 15 cm) of Dowex-1 ($HCOO^-$). The column is eluted with water (200 ml) and then with linear gradient (2 l of water, 2 l of 0·3M formic acid). Two small peaks appear at 0·06M and 0·09M formic acid. The product is eluted with 0·13M formic acid. Fractions are evaporated to a volume of 50 ml and evaporation is continued at 20°C, 1 Torr. The residue is evaporated with 1-butanol (10 ml) and triturated with ether (150 ml). Crystalline substance is collected, washed with ether and dried under diminished pressure. Yield 401 mg (52%), $R_F$ ($S_1$) 0·17. For $C_{10}H_{13}N_8O_7P$ (388·2) calculated: 30·96% C, 3·35% H, 28·80% N, 10·75% P; found: 30·52% C, 3·41% H, 28·12% N, 10·65% P. UV spectrum (water): $\lambda_{max}$ 282 nm, $\lambda_{min}$ 249 nm; $A_{282}/A_{249}$ 3·33; $\varepsilon_{max}$ 14·55 . $10^3$.

B): The stirred suspension of 8-azidoadenosine (6 mmol) in triethyl phosphate is cooled in ice water, phosphoryl chloride (1·2 ml) is added, and the mixture stirred under cooling for 1 h. Ether (230 ml) is added, the precipitate collected by suction, washed with ether (100 ml) and dried under diminished pressure. The substance is dissolved in 2M triethylammonium hydrogen carbonate (25 ml) and, after 20 h at 4°C, evaporated. The residue is evaporated with three 30 ml portions of ethanol, dissolved in water and the product isolated by above described method. Yield 1·98 g (85%).

8-Bromoadenosine (Modification of described procedure[8])

The solution of adenosine (0·1 mol) in warm (45°C) 50% aqueous acetic acid (100 ml) is added to the stirred mixture of water (1 l), sodium acetate (1 mol), acetic acid (50 ml) and bromine (6·1 ml). The mixture is stirred for 3 h. Crystalline precipitate is deposited during this time. A solution of sodium hydrogen sulfite is added to discolour the supernatant and pH is adjusted to 7·5 by 10% aqueous sodium hydroxide. After 1 h at 0°C, the product is collected, washed

with water (300 ml), then with acetone (100 ml) and ether (100 ml) and dried under diminished pressure. Yield 23·2 g (67%), $R_F$ ($S_2$) 0·14.

2',3'-O-Isopropylidene-8-bromoadenosine (*IIIb*)

6M Solution of hydrogen chloride in dimethylformamide (12 ml) is added to the stirred suspension of 8-bromoadenosine (50 mmol) in the mixture of acetone (50 ml), dimethylformamide (100 ml) and 2,2-dimethoxypropane (50 ml). The resulting solution is allowed to stand 18 h. Triethylamine (12 ml) is added and the mixture cooled in ice. Triethylamine hydrochloride is filtered off, washed with dimethylformamide (20 ml) and the filtrate is evaporated. After the removal of volatile solvents, dimethylformamide is evaporated at 40°C, 1 Torr. The residue is dissolved in methanol (50 ml) and ether (50 ml) is added. After 5 h at 0°C, the substance is collected, washed with a mixture of methanol–ether (1 : 1; 30 ml), then with ether and dried under diminished pressure. Yield 11·54 g (60%), $R_F$ ($S_2$) 0·45. The filtrate contains the mixture of *IIIb* and the mixed ketal on $C_5'$-hydroxyl. The solution is evaporated, the residue dissolved in 50% aqueous dioxane and treated with dry ice (~2 g). After 20 h at 0°C, the second crop of *IIIb* is collected (4·2 g; 22%).

2',3'-O-Ethoxymethylene-8-bromoadenosine (*IIIa*)

6M Solution of hydrogen chloride (2·2 ml) is added to the stirred suspension of 8-bromoadenosine (10 mmol) in a mixture of dimethylformamide (15 ml) and ethylorthoformate (5 ml). After 1 h $R_F$ ($S_3$) 0·19 → 0·90. Triethylamine (2·5 ml) is added, the mixture cooled in ice and, after 1 h, filtered. The filtrate is evaporated (35°C, 1 Torr) to a syrupy mixture of diastereoisomers *IIIa* (4·7 g), $R_F$ ($S_2$) 0·65. The product is directly used for the preparation of compound *V*.

2',3'-O-Isopropylidene-8-azidoadenosine (*IVb*)

Substance *IIIb* (1 mmol) and sodium azide (5 mmol) is heated in dimethyl sulfoxide (5 ml) to 80°C 18 h. Chloroform (10 ml) and water (10 ml) are added, the mixture shaken, the chloroform layer extracted with water (5 ml), dried over anhydrous magnesium sulfate and evaporated. The residue is dried at 40°C, 0·1 Torr for several hours to afford *IVb* (224 mg, 58%), m.p. 172—173°C. $R_F$ ($S_2$) 0·45. Analytical sample, crystallized from methanol, has m.p. 178—179°C. For $C_{13}H_{16}N_8O_4$ (348·3) calculated: 44·80% C, 5·09% H, 32·15% N; found: 44·51% C, 4·82% H, 32·81% N.

8-Azidoadenosine[9] (*V*)

The solution of 2',3'-O-ethoxymethylene-8-bromoadenosine (4·7 g) and sodium azide (3·3 g) in dimethyl sulfoxide (50 ml) is heated to 80°C for 18 h. Chloroform (100 ml) and water are added, the mixture shaken, the chloroform layer washed with two 25 ml portions of water, dried over anhydrous magnesium sulfate and evaporated. The residue (*IVa*) is dissolved in 30% aqueous acetic acid (100 ml) and heated to 50° for 2 h. The solution is evaporated, the residue evaporated with toluene (20 ml) and dissolved in ethanol (10 ml). Ether (80 ml) is added and, after 20 h at 0°C, the crystalline *V* is collected, washed with ether and dried under diminished pressure. Yield 1·98 g (65% from *IIIa*). UV spectrum (water): $\lambda_{max}$ 283 nm, $\lambda_{min}$ 252 nm.

*Analyses were performed in the analytical Department (Dr J. Horáček, Head) of this Institute.*

REFERENCES

1. Wagenwoort R. J., Van Der Kraan I., Kemp A.: Biochim. Biophys. Acta *460*, 17 (1977).
2. Haley B. E., Hoffman J. F.: Proc. Nat. Acad. Sci. U.S.A. *71*, 3367 (1974).
3. Cartwright I. L., Hutchinson D. W., Armstrong V. W.: Nucleic Acids Res. *3*, 2331 (1976).
4. Holmes R. E., Robins R. K.: J. Amer. Chem. Soc. *87*, 1772 (1965).
5. Michelson A. M.: Biochim. Biophys. Acta *91*, 1 (1964).
6. Yoshikawa M., Kato T., Takenishi T.: Bull. Soc. Chem. Jap. *42*, 3505 (1968).
7. Ikehara M., Tazawa I., Fukui T.: Chem. Pharm. Bull. *17*, 1019 (1969).
8. Ikehara M., Kaneko M.: Tetrahedron *26*, 4251 (1970).
9. Ikehara M., Uesuki S., Kaneko M.: Chem. Commun. *1967*, 17.

Translated by the author (J. S.).