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A Facile Synthesis of 2-Azidoadenosine Derivatives from Guanosine as Photoaffinity Probes

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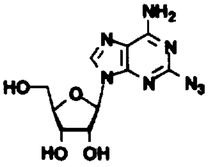
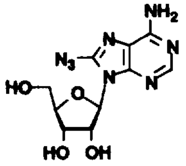
Abstract. 2-Azidoadenosine (**1**) was synthesized in an overall yield of 49% from guanosine *via* the reaction of 9-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-2-amino-6-chloropurine (**2**) with isoamyl nitrite and trimethylsilyl azide under neutral and anhydrous conditions. As photoaffinity probes, ATP analogues and the cap structure of eukaryotic mRNA bearing 2-azidoadenosine were synthesized.

Photoaffinity labelling is a useful tool for the determination of nucleotide binding sites on the nucleotide binding proteins. Particularly, adenosine derivatives as photoaffinity labeling probes are very important, because adenosine derivatives such as ATP and NAD, play an important role in various biological reactions and many proteins are known to be bound to such compounds.

2-Azidoadenosine and 8-azidoadenosine derivatives have been frequently used as photoaffinity labeling probes.[1] 2-Azidoadenosine derivatives usually have higher affinity for several adenine nucleotide binding proteins than 8-azidoadenosine derivatives, because 2-azidoadenosine derivatives prefer the *anti* conformation around the glycosyl bond.[2] On the other hand, 8-azidoadenosine derivatives prefer unusual *syn* conformation owing to steric hindrance of the 8-azido group.[2] However, 2-azidoadenosine derivatives have not been used as often as 8-azidoadenosine derivatives, since the synthesis of 2-azidoadenosine was more difficult than that of 8-azidoadenosine.



1
2-azidoadenosine



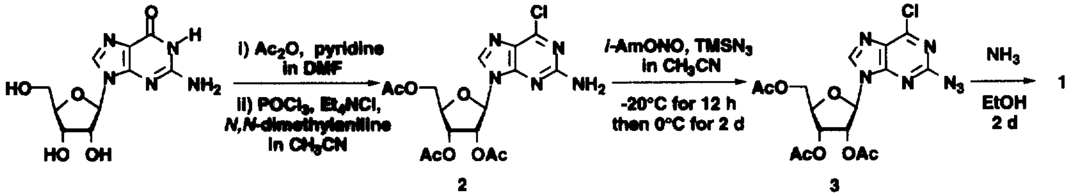
8-azidoadenosine

Previously, 2-azidoadenosine (**1**) has been synthesized by two methods reported by Schaeffer[3] and Nair.[4] The former started from rather expensive 2-chloroadenosine and the latter involved a multi-step procedure with a low yield of **1**. Therefore, development of a new method for the synthesis of **1** is highly desired.

Guanosine seems to be a suitable starting material, because it is transformed easily to the key intermediate, 9-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-2-amino-6-chloropurine (**2**) in 74% yield *via* a 2-step reaction according to the procedure reported previously.[5] The 2-amino group of **2** can be converted into an azido function.

There have been many methods for transformation of the amino function into the azido function *via* diazonium-pentazole.[6-9] Melhado[10] reported that the transformation of aminoindoles into azidoindoles was carried out in aqueous acetic acid by successive treatments with $NaNO_2$ followed by NaN_3. However, when the same procedure was applied to the synthesis of compound **3**, the amino group was converted to an acetoxy group and an only trace amount of 2-azidoadenosine precursor **3** was obtained.

After several screenings, we found that **2** was easily converted to **3**[11] in 75% yield by treatment with 5 equiv each of isoamyl nitrite (*i*-AmONO) and trimethylsilyl azide ($TMSN_3$) in acetonitrile at -20°C for 12 h then at 0 °C for 2 d. Treatment of **3** with NH_3 / ethanol at 30 °C afforded **1**[12] in 89% yield. Thus, **1** was obtained in 49% yield from guanosine *via* the 4-step reaction (Scheme 1).

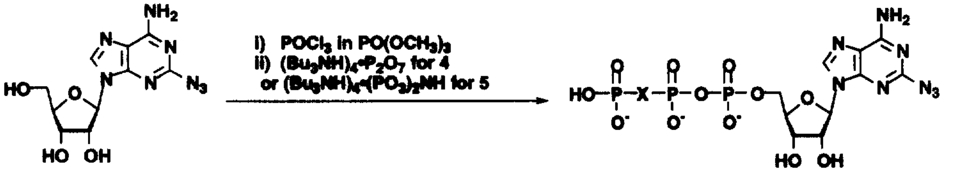


2 **3**

Scheme 1. Synthesis of 2-azidoadenosine (**1**).

Compound **1** was converted to 2-azidoadenosine 5'-triphosphate (**4**)[2] and its β,γ-imidodiphosphate analogue ($2N_3AMP$-PNP, **5**)[13] in one-flask by a modification of the Zimmet's[14] and Yount's[15] procedures in 58 % and 18 % yields, respectively (Scheme 2).

These compounds would be used for photoaffinity labelling of ATPase.



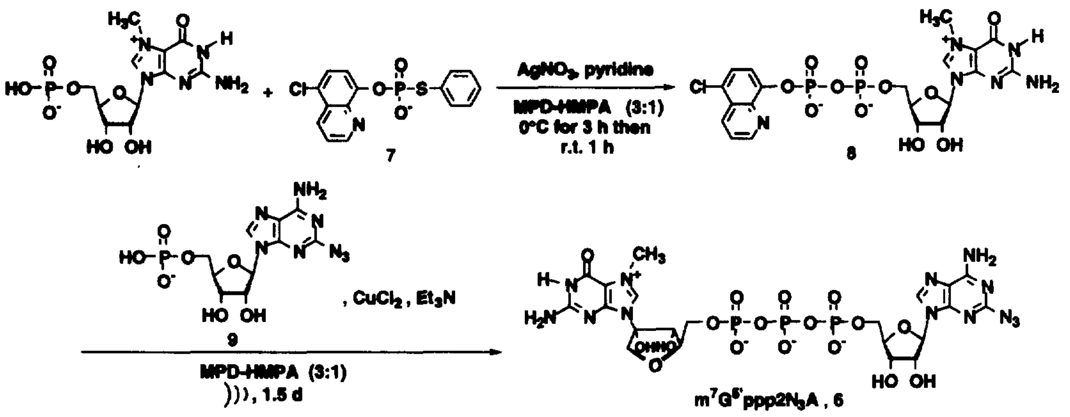
1

4 X = O
5 X = NH

Scheme 2. Synthesis of $2N_3ATP$ (**4**) and $2N_3AMP$-PNP (**5**).

Next, we tried to synthesize an analogue of the cap structure of eukaryotic mRNA, $m^7Gppp2N_3A$ (**6**) using a bifunctional phosphorylating reagent **7**.[16] First, 2-azidoadenosine 5'-phosphate (**8**), prepared in 84% yield from **1** by the Yoshikawa's method,[17] was dissolved in a mixed solvent of 1-methylpyrrolidone (MPD)-HMPA (3:1, v/v). Compound **7** was added to this solution and the phenylthio group was activated by addition of $AgNO_3$. After the formation of the unsymmetric diphosphate, 7-methylguanosine 5'-phosphate and anhydrous $CuCl_2$ were added. However, a 2-aminoadenosine derivative, a reduced species of **6** at the azido group, was obtained instead of the desired product **6**. This result was caused by the reduction of the azido group with benzenethiol, released from **7**, in the presence of copper salts.[18] Therefore, 7-methylguanosine 5'-phosphate was treated with 2 equiv of $AgNO_3$ and 1 equiv of **7** in MPD-HMPA (3:1, v/v) for 3 h at 0°C then 1 h at room temperature. The resulting 'capping reagent' **8** was isolated in 56% yield, then it was allowed to react with 1 equiv of **9** and 3 equiv of

$CuCl_2$ in the presence of 5 equiv of triethylamine in MPD-HMPA (3:1, v/v) for 1.5 d to afford **6**[19] in 61% yield (Scheme 3).



Scheme 3. The synthesis of the cap analogue, $m^7G^{5'}ppp2N_3A$ (**6**).

Similarly, a non-methylated cap analogue, $Gppp2N_3A$ was obtained in 48% yield from guanosine 5'-phosphate, **3** and **7**. This cap analogue could be also converted to **6** by methylation with methyl methanesulfonate (MMS) in DMSO.[20] The ^{14}C-labeled photoaffinity labeling reagent **6** of the cap analogue might be obtained, if $Gppp2N_3A$ would be methylated with ^{14}C-labeled MMS.

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11. Compound **2** (0.856 g, 2.00 mmol) was put in a 50 ml 2-necked round flask, equipped with a three-way cock, a balloon and a septum cap, and dissolved in 20 ml of dry acetonitrile. The system was purged by Ar. This solution was cooled to -20°C on an ice-salt bath, then $TMSN_3$ (1.33 ml, 10 mmol) and *i*-AmONO (1.34 ml, 10 mmol, synthesized according to almost the same procedure as described in *Org. Synth. Coll. Vol. 2*; 108-109 and fleshly distilled under Ar at 1 atm) were added. The mixture was allowed to stand in a freezer (-20°C) for 12 h, then stored in a refrigerator (0°C) for 1 d. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (35~40% AcOEt in hexane) to give 0.687 g (75%) of **3** as amorphous solid. IR (KBr): cm^{-1} 2140, 2155 (N_3); ^1H-NMR ($CDCl_3$, 270 MHz) δ

8.26 (s, 1H, 8-H), 6.20 (d, 1H, J = 4.95 Hz, 1'-H), 5.85 (dd, 1H, J = 5.65, 5.28 Hz, 3'-H), 5.60 (dd, 1H, J = 5.28, 4.95 Hz, 2'-H), 4.48 (m, 1H, 4'-H), 4.41 (m, 2H, 5'-H) 2.17, 2.14, 2.10 (3s, 9H, 2', 3', 5'-CH_3); ^{13}C-NMR ($CDCl_3$, 67.8 MHz) δ 170.28, 169.58, 169.38, 156.49, 152.76, 143.21, 129.41, 86.58, 80.41, 73.12, 70.37, 62.89, 20.75, 20.52, 20.38; MS (FAB) 454 ($M+H^+$), 259 (M-B), 196 ($B+2H^+$).

12. Compound 3 (1.54 g, 3.39 mmol) was dissolved in 40 ml of dry ethanol in a 100 ml round flask. This solution was cooled to -15°C on an ice-salt bath and dry NH_3 gas was passed through this solution for 1 h. This flask was sealed tightly and stirred at 30°C. After 2 d, the solution was evaporated (CAUTION! Cool it before open) and the residue was dissolved in a small amount of hot methanol. Then hot water was added and the solution was allowed to stand at 4°C. Compound 1 was crystallized and separated by filtration. It was dried *in vacuo* at 100°C to give 0.771 g (73%) of 1. The filtrate was evaporated under reduced pressure to dryness and the residue was purified by silica gel column chromatography (7~10% MeOH in CH_2Cl_2) to give 0.166 g (16%) of 1 as second crop. The NMR spectra of 1 have not yet been reported. We observed two sets of signals corresponding to each for azidomethine and tetrazole forms instead of three sets of signals as previously reported for 2-azidoadenine.[2,21] The azidomethine form: ^{1}H-NMR (DMSO-d_6, 270 MHz) δ 8.39 (s, 1H, 8-H), 7.74 (br, 2H, 6-NH_2), 5.86 (d, 1H, J = 5.94 Hz, 1'-H), 5.55 (d, 1H, J = 6.27 Hz, 2'-OH), 5.30 (d, 1H, J = 4.62 Hz, 3'-OH), 5.17 (dd, 1H, J = 5.61, 5.28 Hz, 5'-OH), 4.60 (m, 1H, 2'-H), 4.23 (d, 1H, J = 4.62 Hz, 3'-H), 4.02 (m, 1H, 4'-H), 3.5~3.8 (m, 2H, 5'-H); ^{13}C-NMR (DMSO-d_6, 67.8 MHz) δ 156.68, 155.58, 150.57, 139.64, 116.97, 87.46, 85.73, 73.44, 70.57, 61.60. The tetrazole form: ^{1}H-NMR (DMSO-d_6, 270 MHz) δ 9.54 (br, 2H, 6-NH_2), 8.67 (s, 1H, 8-H), 6.05 (d, 1H, J = 5.28 Hz, 1'-H), 5.63 (d, 1H, J = 5.94 Hz, 2'-OH), 5.33 (d, 1H, J = 4.95 Hz, 3'-OH), 5.24 (dd, 1H, J = 5.61, 5.28 Hz, 5'-OH), 4.60 (m, 1H, 2'-H), 4.27 (d, 1H, J = 4.29 Hz, 3'-H), 4.02 (m, 1H, 4'-H), 3.5~3.8 (m, 2H, 5'-H); ^{13}C-NMR (DMSO-d_6, 67.8 MHz) δ 153.78, 152.24, 142.84, 141.08, 111.54, 87.46, 85.48, 73.61, 70.23, 61.24.
13. Compound 5 was synthesized by almost the same protocol as reported in ref. 15 except for using imidodiphosphate (7.5 equiv) instead of pyrophosphate. Tri-*n*-butylammonium imidodiphosphate solution was also prepared according to ref. 15 using 4 equiv of tri-*n*-butylamine, evaporated *in vacuo* under 30°C, with 87 % purity estimated by ^{31}P-NMR. Compound 5 also indicated 2 sets of ^{1}H-NMR spectra due to the azidomethine-tetrazole equilibrium of the 2-azidoadenosine moiety. ^{1}H-NMR (D_2O, 270 MHz) δ 8.57, 8.41 (2s, 1H, 8-H), 6.17, 6.02 (2d, 1H, 1'-H), 4.73 (m, 1H, 2'-H), 4.55 (m, 1H, 3'-H), 4.36 (m, 1H, 4'-H), 4.23 (m, 2H, 5'-H); ^{31}P-NMR (D_2O, 109 MHz) δ -0.82 (brs, 1P, P^3), -10.19 (m, 2P, P^1 and P^2). ^{31}P-chemical shifts were given relative to 85 % H_3PO_4 as an external standard.
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19. Compound 6 also has 2 sets of ^{1}H-NMR spectra due to the azidomethine-tetrazole equilibrium of the 2-azidoadenosine moiety: ^{1}H-NMR (D_2O, 270 MHz) δ 8.45, 8.19 (2s, 1H, 8-H of $2N_3A$), 6.01, 5.83, 5.77, 5.74 (4d, 2H, 1'-H's), 4.7~4.1 (m, 10H, 2', 3', 4', and 5'-H's), 3.97, 3.94 (2s, 3H, CH_3 of m^7G); ^{31}P-NMR (D_2O, 109 MHz) δ -10.94 (d, 2P, $^2J_{PP}$ = 16.78 Hz, P^1 and P^3), -22.43, -22.49 (2t, 1P, $^2J_{PP}$ = 19.07 Hz, P^2). ^{31}P-chemical shifts were given relative to 85 % H_3PO_4 as an external standard.
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