SYNTHESIS OF N,N'-[BIS(ADENOSIN-8-YL)]DIAMINOALKANES AND -PIPERAZINE

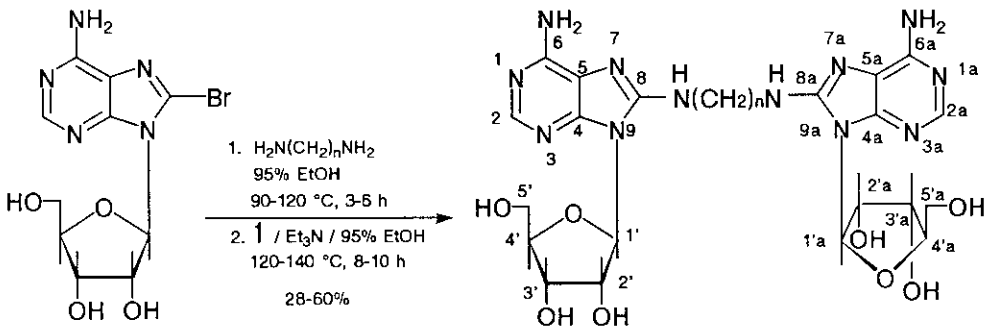
Yuris A. Maurinsh,[1] Keiko Sujino, Hideo Togo, and Masataka Yokoyama*
Department of Chemistry, Faculty of Science, Chiba University, Yayoi-cho, Chiba City 260, Japan

Abstract —— Several N,N'-[bis(adenosin-8-yl)]diaminoalkanes and -piperazine were prepared by the following procedure; 8-bromoadenosine reacted with diamines to give the corresponding N-(adenosin-8-yl)diaminoalkanes and -piperazine, which were then converted into the title compounds by treatment with another 8-bromoadenosine.

Polyamines are present in all mammalian cells and known to be important for DNA replication, cell differentiation, and growth regulation.[2] Deranged polyamine metabolism may be an essential factor in cancerogenesis. Inhibitors of polyamine biosynthesis might be useful therapeutic agents in a variety of diseases including cancer.[3] As it is known that polyamines can interact with DNA and RNA chains through electrostatic interactions between the protonated N atoms in the amine molecules and the phosphate groups of the nucleotides[4] and as DNA molecules containing polyamines have been isolated from bacteriophages,[5] it seemed interesting to synthesize nucleosides bearing polyamine residues.

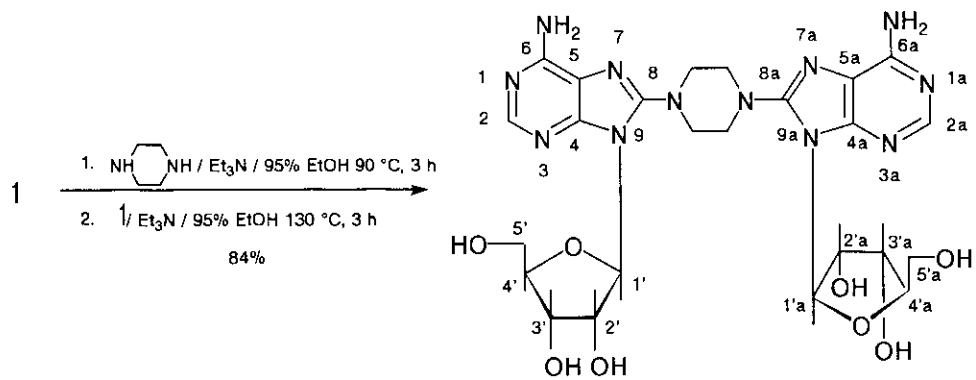
In 1973, the synthesis of some deoxyoligonucleotides containing N-thyminylputrescine was reported.[6] Some 8-(ω-aminoalkylamino)adenosine derivatives were also synthesized.[7~9] While, during our investigation on the synthesis of 8-(ω-aminoalkylamino)adenosine, -inosine, and -guanosine,[10] we noticed that besides the desired 8-(ω-aminoalkylamino)nucleosides some amounts (5~15% yields) of N,N'-[bis(adenosin-8-yl)]diaminoalkanes like 2a~c were present in the reaction mixtures

(checked by hplc). Herein we wish to report on the synthesis of hitherto unknown type of N,N'-[bis(adenosin-8-yl)]diaminoalkanes and -piperazine. The following principal method for the preparation of these compounds is recommended: Reaction of 8-bromoadenosine 1 with an excess of diamine to prepare mainly monosubstituted diamines; after removal of the excess diamine, further reaction of monosubstituted derivatives with an equimolar amount of 1 to give N,N'-[bis(adenosin-8-yl)]diaminoalkanes 2a~c and -piperazine 3.



1

2a, n=3
2b, n=4
2c, n=5



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The first step of the reactions using $H_2N(CH_2)_nNH_2$ (n = 3,4,5) and piperazine proceeded smoothly to give the corresponding N-(adenosin-8-yl)diaminoalkanes and -piperazine (checked by tlc). The addition of the second nucleoside moiety to the resulting nucleoside required more vigorous reaction conditions (higher temperature and longer reaction time, see experimental). Our attempts to obtain bis(guanosin-8-yl)diamines or N-(adenosin-8-yl)-N'-(guanosin-8-yl)diamines were unsuccessful even at 160 °C for longer reaction time.

Disubstitution of the diamines were unequivocally established by ^{1}H-nmr spectra showing two sets of signals due to the nucleoside moieties, because the disubstituted diamines were not symmetrical from the small differences in chemical shifts both in ^{1}H- and ^{13}C-nmr spectra.

For the determination of conformation about the glycosyl bond (syn and anti conformer), we used a correlation between the ^{13}C-nmr chemical shifts for C-2' and C-3' which was postulated recently.[11] It was reported that purine nucleosides in syn conformation had usually the difference in chemical shift, Δ at C-2' and C-3' positions <0.5 ppm. In our case, all the synthesized nucleosides had the values of Δ at C-2' and C-3' positions = 0.17~0.26 ppm. It shows that these nucleosides exist in syn conformation predominantly. Further, the existence of this conformation could be supported by their NOESY spectra of ^{1}N-nmr, i.e., the nOe's were observed between H-1' (1'a) and NH of diamine in 2a~c, and between H-1' and CH_2 of piperazine in 3 as well as between OH (C-2') or OH (C-3') and CH_2 of diamines or piperazine.

For estimation of the population of the ribose ring conformations, we used Karplus relationship[12] by which the population (%) of S-type (C-2' endo) conformer could be calculated as % S = 10 X coupling constant ($J_{1',2'}$). Judging from observed $J_{1',2'}$ values (2a: $J_{1',2'}$ = 7.3 Hz; 2b: $J_{1',2'}$ = 7.5 Hz; 2c: $J_{1',2'}$ = 7.3 Hz; 3: $J_{1',2'}$ = 7.3 Hz), the conformer populations of nucleosides (2a~c and 3) are estimated as 73~75% S (C-2' endo).

In a bioassay test, the N,N'-[bis(adenosin-8-yl)]diaminoalkanes and -piperazine synthesized here did not show any growth inhibitory effect against KB nasopharyngeal carcinoma cells (2a~c and 3: ED_{50}= 10μg/ml; cf. 5-fluorouracil: ED_{50}= 0.7μg/ml and adriamycin: ED_{50}= 0.008μg/ml).

EXPERIMENTAL

Microanalysis was performed at the Chemical Analysis Center of Chiba University using a Perkin-Elmer elemental 240 analyser. Ir, ms, uv, and ^{1}H- and ^{13}C-nmr spectra were measured with Hitachi 215, JEOL JMS-DX303, Hitachi EPS-3T, and JEOL JNM-GSX-400 spectrometers, respectively. Optical rotations were measured with a JASCO DIP-370 polarimeter. Hplc was performed by a Shimadzu LC-6A liquid chromatography. Wakogel C-200 was used for low pressure liquid chromatography and Wakogel B-5F was used for tlc.

N,N'-[Bis(adenosin-8-yl)]diaminopropane (2a) A mixture of 1,3-diaminopropane (1.46 ml, 17.4 mmol), 8-bromoadenosine[13] (1, 1.2 g, 3.5 mmol), and 95% aqueous EtOH (20 ml) was heated with stirring in a sealed tube (volume, 50 ml) at 90 °C for 6 h. The reaction mixture was concentrated to a small volume, and Et_2O was added to the residue to remove the excess 1,3-diaminopropane. After stirring for 10 min, the solvents were discarded by decantation, and then oily residue was dissolved in EtOH (5 ml) containing a small amount of Et_3N. Then, Et_2O was added to deposit a semi-solid. The solid was isolated by decantation and purified by successive addition of EtOH and Et_2O.

A mixture of the solid thus obtained, 8-bromoadenosine[13] (1, 1.2 g, 3.5 mmol), Et_3N (2.4 ml, 17.4 mmol), and 95% aqueous EtOH (20 ml) was heated with stirring in the sealed tube at 120 °C for 10 h. After cooling, water (20 ml) was added to cause precipitation of 2a. The precipitate was collected by filtration and recrystallized from H_2O and then from EtOH to give 2a; 51% yield; white powder (dried over $CaCl_2$); mp: 221~222 °C; R_f [i-PrOH/aqueous NH_3 (25%)/H_2O = 7 : 1 : 2) = 0.58; $[\alpha]_D^{20}$ -32.8° (c = 0.08, EtOH). Anal. Calcd for $C_{23}H_{32}N_{12}O_8.3.5H_2O$: C, 41.38; H, 5.89; N, 25.18. Found: C, 41.18; H, 5.68; N, 25.12. Ms m/z 605 [positive fabms, $(M + 1)^+$], 603 [negative fabms, $(M - 1)^-$]. Uv (99% aqueous EtOH: λ_{max} 279 nm (ε 26600), 239 nm (ε 3500). Ir (KBr) : ν 3250 (NH, NH_2, OH), 2900 cm^{-1} (CH, CH_2). ^{1}H-Nmr (DMSO-d_6): δ 1.95 (t, J = 6.8 Hz, 2H, CH_2), 3.45 (m, 4H, 2 x N-CH_2), 3.66 (m, 4H, 4 x H-5'), 3.99 (s, 2H, 2 x H-4'), 4.15 (s, 2H, 2 x H-3'), 4.71 [dd, J = 7.3 Hz, 6.7 Hz, 2H, H-2' (2'a)], 5.2 (d, J = 4.0 Hz, 2H, 2 x OH), 5.29 (d, J = 6.6 Hz, 2H, 2 x OH), 5.9 (d, J = 7.3 Hz, 2H, 2 x H-1'), 5.98(pseudo t, J = 5.0 Hz, 2H, 2 x OH), 6.49 (s, 4H, 2 x NH_2), 6.98 (t, J = 4.8 Hz, 2H, 2 x NH), 7.91 (s, 2H, 2 x H-2). ^{13}C-Nmr (DMSO-d_6): δ 28.67 (CH_2), 39.75 (2 x N-CH_2), 61.73 (2 x C-5'), 70.81 (2 x C-3'), 70.98 (2 x C-2'), 85.74 (2 x

C-4'), 86.51 (2 x C-1'), 117.0 (2 x C-5), 148.56 (2 x C-2), 149.71 (2 x C-8), 151.45 (2 x C-4), 152.32 (2 x C-6).

N,N'-[Bis(adenosin-8-yl)]diaminobutane (2b) A mixture of 1,4-diaminobutane (1.5 ml, 14.5 mmol), 8-bromoadenosine[13] (1, 1.0 g, 2.9 mmol), and 95% aqueous EtOH (20 ml) was worked up in the same way as that described for 2a (first step: 110 °C, 5 h; second step: 130 °C, 8 h). Recrystallization from H_2O gave 2b; addition of charcoal was needed for decolorization; 0.5 g (28%, based on the starting amount of 1); white powder (dried over $CaCl_2$); mp: 223~224 °C: R_f (i-PrOH/aqueous NH_3 (25%)/H_2O = 7 : 1 : 2) = 0.45; $[\alpha]_D^{20}$ -50.5° (c = 0.02, DMSO). Anal. Calcd for $C_{24}H_{34}N_{12}O_8.2H_2O$: C, 44.03; H, 5.85; N, 25.67. Found: C, 44.26; H, 6.02; N, 25.94. Ms m/z 619 [positive fabms, $(M + 1)^+$], 617 [negative fabms, $(M - 1)^-$]. Uv (99% aqueous EtOH): λ_{max} 280 nm (ε 20600), 241 nm (ε 6800). Ir (KBr): ν 3250 (NH, NH_2, OH), 2900, 2840 cm^{-1} (CH, CH_2). ^{1}H-Nmr (DMSO-d_6): δ 1.68 (s, 4H, 2 x CH_2), 3.37 (m, 4H, 2 x N-CH_2), 3.62 (m, 4H, 4 x H-5'), 3.97 (s, 2H, 2 x H-4'), 4.25 (br s, 2H, 2 x H-3'), 4.67 (dd, J = 7.5 Hz, 7.0 Hz, 2H, 2 x H-2'), 5.17 (d, J = 4.3 Hz, 2H, 2 x OH), 5.26 (d, J = 6.3 Hz, 2H, 2 x OH), 5.89 (d, J = 7.5 Hz, 4H, 2 x OH, 2 x H-1'), 6.48 (s, 4H, 2 x NH_2), 6.93 (t, J = 4.8 Hz, 2H, 2 x NH), 7.90 (s, 2H, 2 x H-2). ^{13}C-Nmr (DMSO-d_6): δ 26.37 (2 x CH_2), 42.10 (2 x N-CH_2), 61.59 (C-5'), 61.69 (C-5'a), 70.73 (2 x C-3'), 70.95 (2 x C-2'), 85.68 (2 x C-4'), 86.35 (2 x C-1'), 117.05 (C-5), 117.08 (C-5a), 148.41 (2 x C-2), 149.73 (2 x C-8), 151.32 (C-4), 151.38 (C-4a), 152.22 (C-6), 152.27 (C-6a).

N,N'-[Bis(adenosin-8-yl)]diaminopentane (2c) A mixture of 1,5-diaminopentane [prepared in situ from 1,5-diaminopentane hydrochloride (2.5 g, 14.5 mmol) and two molar equivalents of NaOEt], 8-bromoadenosine[13] (1, 1.0 g, 2.9 mmol), EtOH (20 ml), and H_2O (1 ml) was worked up in the same way as described above for 2a (first step: 120 °C, 3 h; second step: 140 °C, 8 h). Recrystallization from H_2O gave 2c; 1.1 g (60%, based on the starting amount of 1); white powder (dried over $CaCl_2$); mp: 118~119 °C; R_f (i-PrOH/aqueous NH_3 (25%)/H_2O = 7 : 1 : 2) = 0.68; $[\alpha]_D^{20}$ -34.3° (c = 0.03, DMSO). Anal. Calcd for $C_{25}H_{36}N_{12}O_8.3H_2O$: C, 43.73; H, 6.17; N, 24.48. Found: C, 43.93; H, 6.19; N, 24.70. Ms m/z 633 [positive fabms, $(M + 1)^+$], 631 [negative fabms, $(M - 1)^-$]. Uv (99% aqueous EtOH): λ_{max} 280 nm (ε 32500), 239 nm (ε 2700). Ir (KBr): ν 3270 (NH, NH_2, OH), 2880, 2810 cm^{-1}

(CH, CH_2). ^{1}H-Nmr (DMSO-d_6):δ 1.41 (m, 2H, CH_2), 1.65 (m, 4H, 2 x CH_2), 3.35 (m, 4H, 2 x N-CH_2), 3.65 (m, 4H, 4 x H-5'), 3.98 (d, J = 2.0 Hz, 2H, 2 x H-4'), 4.13 (d, J = 0.5 Hz, 2H, 2 x H-3'), 4.65 (t, J = 4.0 Hz, 2H, 2 x H-2'), 5.16 (br s, 2H, 2 x OH), 5.23 (br s, 2H, 2 x OH), 5.91 (d, J = 7.3 Hz, 4H, 2 x OH, 2 x H-1'), 6.46 (s, 4H, 2 x NH_2), 6.89 (t, J = 6.0 Hz, 2H, 2 x NH), 7.90 (s, 2H, 2 x C-2). ^{13}C-Nmr (DMSO-d_6):δ 23.79 (CH_2), 28.37 (2 x CH_2), 42.22 (2 x N-CH_2), 61.59 (2 x C-5'), 70.63 (2 x C-3'), 70.89 (2 x C-2'), 85.59 (2 x C-4'), 86.23 (2 x C-1'), 116.95 (2 x C-5), 148.33 (2 x C-2), 149.66 (2 x C-8), 151.25 (2 x C-4), 152.17 (2 x C-6).

N,N'-[Bis(adenosin-8-yl)]piperazine (3) A mixture of piperazine hexahydrate (2.8 g, 14.5 mmol), Et_3N (2.0 ml, 14.5 mmol), 8-bromoadenosine[13] (1, 1.0 g, 2.9 mmol), and 95% aqueous EtOH (20 ml) was worked up in the same way as described above for 2a (first step: 90 °C, 3 h; second step: 130 °C, 3 h). The gelatinous residue thus obtained was coevaporated with dry benzene to remove a trace of water and recrystallized from DMF-AcOEt to give 3; 1.5 g (84%, based on the starting amount of 1); white powder (dried over $CaCl_2$); mp: 181~182 °C(dec.); R_f (i-PrOH/aqueous NH_3 (25%)/H_2O = 7 : 1 : 2) = 0.55; $[\alpha]_D^{20}$ -10.5° (c = 0.05, DMSO). Anal. Calcd for $C_{24}H_{32}N_{12}O_8 \cdot 6H_2O$: C, 39.78; H, 6.12; N, 23.19. Found: C, 39.43; H, 5.98; N, 23.13. Ms m/z 617 [positive fabms, $(M + 1)^+$], 615 [negative fabms, $(M - 1)^-$]. Uv (99% aqueous EtOH): λ_{max} 273 nm (ε 23100); 240 nm (ε 3600). Ir (KBr): ν 3500 cm^{-1} (NH_2, OH). ^{1}H-Nmr (DMSO-d_6):δ 3.20~3.50 (m, 8H, 4 x CH_2), 3.60 (m, 4H, 4 x H-5'), 3.92 (s, 2H, 2 x H-4'), 4.13 (s, 2H, 2 x H-3'), 4.97 [br s, 1H, H-2 (2'a)], 5.03 [br s, 1H, H-2'a (2')]; 5.15 (br, 2H, 2 x OH), 5.38 (br s, 2H, 2 x OH), 5.64 (d, J = 7.3 Hz, 1H, H-1'), 5.69 (br d, J = 7.3 Hz, 3H, 2 x OH, H-1'a), 7.00 (s, 2H, NH_2), 7.07 (s, 2H, NH_2), 7.99 (s, 2H, H-2). ^{13}C-Nmr (DMSO-d_6):δ 42.54 (CH_2), 45.72 (CH_2), 48.00 (CH_2), 50.53 (CH_2), 62.25 (C-5'), 62.32 (C-5'a), 70.95(2 x C-3'), 71.13 (2 x C-2'), 86.45 (C-4'), 86.73 (C-4'a), 88.00 (2 x C-1'), 116.68 (C-5), 116.88 (C-5a), 149.04 (C-8), 149.16 (C-8a), 150.71 (C-2), 150.99 (C-2a), 153.75 (2 x C-4), 154.63 (C-6), 154.80 (C-6a).

Bioassay KB cells (2 x 10^3/well/0.1 ml) were seeded into 96 well mutiplates. One day after the start of culture, 0.1 ml of culture medium containing a test drug dissolved in DMSO at various concentrations was added to the wells. Final

concentration of DMSO was lower than 0.1%. Three days after the drug application, cell numbers were estimated by the dye-staining method.[14] The ED_{50} values are the concentrations of the samples that reduce proliferation rates to 50% of that of untreated cultures.

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