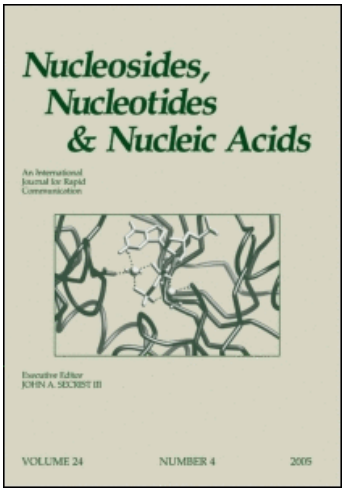

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SYNTHESIS OF 8-SUBSTITUTED ANALOGS OF 3'-DEOXY-3'-FLUOROADENOSINE[1)]

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ABSTRACT: After 2',5'-di-*O*-protection of 8-bromoadenosine, the product was converted to the xyloside, which was successively treated with diethylaminosulfur trifluoride (DAST) and acid to afford 8-bromo-3'-deoxy-3'-fluoroadenosine. 8-Mercapto and 8-oxy analogs were obtained from 8-bromo congener.

Recent development of biology revealed that the 2',5'-linked oligomers are biologically important. For instance, 2',5'-oligoadenylate plays a key role in the antiviral action of interferon.[2)] Poly (ADP-ribose) composed of 2'-*O*-ribosylated ADP is also recognized as a polymer required for the process of cellular recovery from DNA damage.[3)] In this connection, 2',5'-oligoadenylates containing a modified sugar[4)] or base[5)] have been synthesized and their physico-chemical and biological properties have been studied. These nucleosides benefit from greater chemical or metabolic stability than their ribofuranose counterparts and many of them are endowed with remarkable biological activities. Therefore, we planned to explore the biological activity of 2',5'-oligoadenylates containing adenosine modified both sugar and base moieties. In this paper, a method for the synthesis of 8-substituted analogs of 3'-deoxy-3'-fluoroadenosine is described.

There have been reports on the chemical synthesis of nucleosides fluorinated in the sugar moiety.[6)] Morizawa *et al.*[7)] reported the preparation of 3'-deoxy-3'-fluoro-adenosine using a glycosidation method. Recently Herdewijn *et al.*[6)] synthesized 3'-deoxy-3'-fluoroadenosine by the reaction of diethylaminosulfur trifluoride (DAST) with

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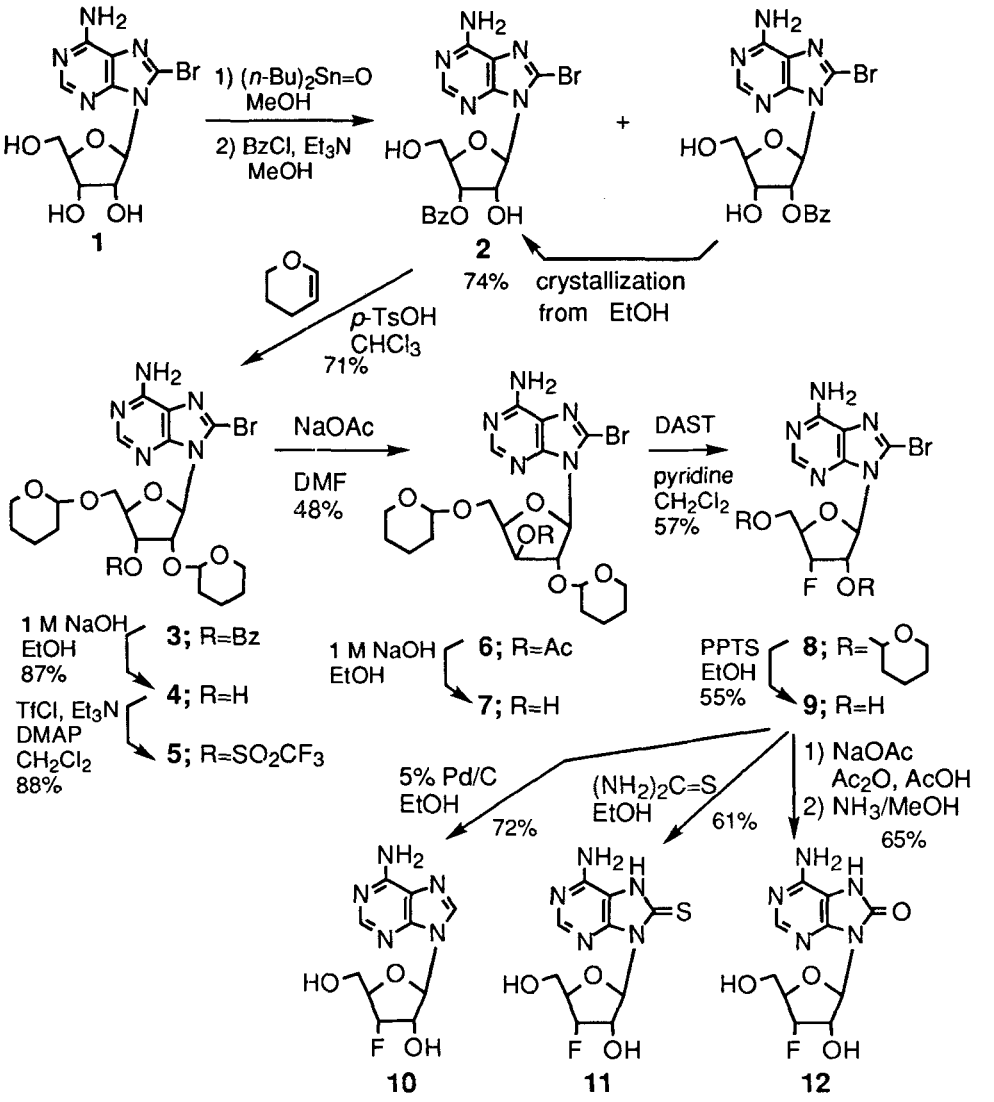
This paper is dedicated to the late Professor Tsujiaki Hata who made a great contribution to nucleic acid chemistry.

the xyloside. Although we adopted this approach for the synthesis of 8-bromo congener, the synthetic pathway to obtain the xyloside was modified for a large scale reaction as following. 2',3'-*O*-Di-*n*-butylstannylene complex is a useful intermediate for the introduction of only one benzoyl group onto the 2'-OH and/or 3'-OH.[8] Thus, 8-bromo-adenosine **1**[9] was successively treated with di-*n*-butyltin oxide and excess benzoyl chloride in the presence of triethylamine followed by recrystallization from EtOH after usual work-up of the reaction mixture to afford **2** as pale yellowish crystals. The ^{1}H-NMR spectrum of the product revealed that the signal of 3' proton appeared relatively low-field (5.62 ppm) and two protons exchangeable with deuterium oxide were identified as 2'-OH and 5'-OH, indicating that the benzoyl group was introduced at the 3'-OH. Reaction of **2** with 3,4-dihydro-(2*H*)-pyran followed by alkaline hydrolysis of the product gave the 2',5'-di-*O*-(tetrahydropyran-2-yl) derivative **4**. The configuration of the 3'-OH was reversed by treatment with trifluoromethanesulfonyl (triflyl) chloride, nucleophilic displacement with sodium acetate and alkaline hydrolysis to give the xyloside **7**. Fluorination of **7** with DAST was achieved in the presence of pyridine in CH_2Cl_2 as reported earlier.[10] The reaction mixture was subjected to usual work-up to give a sole product **8** in 57% yield, which was deprotected with pyridinium *p*-toluenesulfonate (PPTS) to give 8-bromo-3'-deoxy-3'-fluoroadenosine (**9**), a key intermediate for the synthesis of 8-substituted analog of 3'-deoxy-3'-fluoroadenosine. The ^{1}H-NMR spectrum of **9** indicated that the 3'-fluorine caused a downfield shift of the 3'-proton as well as a large H3'-C-F geminal coupling (52.3Hz). It was difficult to assign the stereochemistry of the product by the two-dimensional NOE (NOESY) experiment. Thus, compound **9** was converted to the known product **10** by hydrogenation with 5% Pd/C under H_2 atmosphere, of which ^{1}H-NMR spectrum was identical in all respects to the published data.[6),7)] Therefore, the structure of **9** was unequivocally determined. Nucleophilic substitution of 8-bromo function of **9** with some nucleophiles afforded the 8-substituted congeners **11**, **12**.

EXPERIMENTAL

Melting points (mp) were determined using a Yanagimoto micro-melting point apparatus (hot stage type) without correction. UV spectra were recorded with a Shimadzu UV-190 digital spectrometer. Low-resolution mass spectra were obtained on a JEOL JMS-AX500 mass spectrometer in the direct-inlet mode. ^{1}H-NMR spectra were recorded on a Varian UNITY 200 (200 MHz) or UNITY 600 (600 MHz) in $CDCl_3$ (or dimethyl sulfoxide (DMSO)-d_6) with tetramethylsilane as an internal standard. Merck Art 5554 plates precoated with silica gel 60 containing the fluorescent indicator F_{254} were used for thin-layer chromatography and silica gel 60 (Merck 7734, 60 - 200 mesh) was employed for column chromatography.



SCHEME

3'-*O*-Benzoyl-8-bromoadenosine (2). A solution of 8-bromoadenosine (**1**)[11] (58.6 g, 0.169 mol) and di-*n*-butyltin oxide 42.75 g (1 eq) in MeOH (2 l) was refluxed for 4 h to give a pale yellowish precipitate. After ice-cooling, triethylamine (202 ml) was added to the mixture, then benzoyl chloride (167 ml, 1.22 mol) was added dropwise for 30 min. The solution was stirred at room temperature for 30 min and concentrated to give a syrup to which hexane (1.5 l) was added. The organic layer was removed by decantation and the residue was washed with hexane (0.5 l), then suspended in water (2.0 l). After stirring for 1 h, the aqueous later was decanted to give a gum, which was washed with water and dissolved in EtOH (500 ml) to afford pale brownish crystals 56.36 g (74%). mp: 204-207℃. UV λmax (MeOH) 265 nm, λmax (0.1N HCl) 263.5 nm, λmax (0.1N NaOH) 265 nm. ^{1}H-NMR (DMSO-d_6) δ: 8.17 (1H, s, H2), 7.65 (2H, br s, NH_2), 7.57-7.73 (5H, m, C_6H_5-), 5.96 (2H, m, 2'-OH, H1'), 5.78 (1H, dd, J = 4.4 Hz, 5'-OH), 5.62 (1H, dd, J = 2.2, 3.7 Hz, H3'), 5.41 (1H, q, J = 6.1 Hz, H2'), 4.36 (1H, m, H4'), 3.78 (1H, m, H5'a), 3.70 (1H, m, H5'b). MS *m/z*: 450, 452 (M^++1). *Anal.* Calcd for $C_{17}H_{16}BrN_5O_5 \cdot 0.5H_2O$: C, 44.46; H, 3.73; N, 15.25. Found: C, 44.57; H, 3.55; N, 15.16.

3'-*O*-Benzoyl-8-bromo-2',5'-di-*O*-(tetrahydropyran-2-yl)adenosine (3). Dried *p*-toluenesulfonic acid (P_2O_5, 1 mmHg, rt, 5.3 g) was added to a solution of **2** (5.0 g, 11.1 mmol) in a mixture of 3,4-dihydro-2*H*-pyran (8.5 ml) and DMF (80 ml) and stirred at room temperature for 3 h, then neutralized with triethylamine. The solution was evaporated *in vacuo* to give a syrup, which was dissolved in benzene (300 ml) and the organic layer was washed with water twice (200 ml), dried over $MgSO_4$. After concentration to a small volume, the residue was chromatographed over a column of silica gel 60 (4.2×40 cm) with 0 - 5% EtOH in hexane (4 l) to give a caramel (6.08 g, 89%). UV λmax (MeOH) 264 nm, λmax (0.1N HCl) 263 nm, λmax (0.1N NaOH) 264 nm. MS *m/z*: 618, 620 (M^++1). *Anal.* Calcd for $C_{27}H_{32}BrN_5O_7 \cdot H_2O$: C, 50.95; H, 5.38; N, 11.00. Found: C, 50.72; H, 5.05; N, 10.71.

8-Bromo-2',5'-di-*O*-(tetrahydropyran-2-yl)adenosine (4). 1M NaOH (40 ml) was added dropwise to a solution of **3** (16.9 g, 27.3 mmol) in EtOH (158 ml), then stirred at room temperature for 30 min. After addition of acetic acid (2.4 ml), the solution was evaporated to give a residue, to which water (200 ml) was added. The solution was extracted from $CHCl_3$ twice (400 ml) and the organic layer was washed with water (200 ml), dried over $MgSO_4$ and concentrated to a small volume. The solution was chromatographed over a column of silica gel 60 (4.2×40 cm) with 0 - 5% EtOH in $CHCl_3$ (4 l) to give a caramel (12.24 g, 87%). UV λmax (MeOH) 264 nm, λmax (0.1N HCl) 263 nm, λmax (0.1N NaOH) 264 nm. MS *m/z*: 514, 516 (M^++1), 428, 430 (M^+ - C_5H_9O). *Anal.* Calcd for $C_{20}H_{28}BrN_5O_6$: C, 46.70; H, 5.49; N, 13.62. Found: C, 46.66; H, 5.44; N, 13.24.

8-Bromo-2',5'-di-*O*-(tetrahydropyran-2-yl)-3'-*O*-trifluoromethanesulfonyladenosine (5). Trifluoromethanesulfonyl chloride (2.0 ml) was added to an ice-cooled solution of **4** (6.18 g, 12.0 mmol) and *N*,*N*-dimethylaminopyridine (1.5 g) in the mixture of Et_3N (1.9 ml, 1.14 eq) and CH_2Cl_2 (60 ml), then the mixture was stirred at room temperature for 40 min. The solution was diluted with $CHCl_3$ and washed with ice-water (100 ml) twice (200 ml), dried over $MgSO_4$ and concentrated to a small volume. The residue was chromatographed over a column of silica gel 60 (4.0×35 cm) with 0 - 5% EtOH in $CHCl_3$ (4 l) to give the trifluoromethanesulfonate (**5**) as a caramel (6.84 g, 88%). UV λmax (MeOH) 263 nm, λmax (0.1N HCl) 263 nm, λmax (0.1N NaOH) 263 nm. *Anal.* Calcd for $C_{21}H_{27}BrF_3N_5O_8S \cdot CF_3SO_3H$: C, 33.17; H, 3.54; N, 8.79. Found: C, 33.51; H, 3.36; N, 8.77.

9-[3-*O*-Acetyl-2,5-di-*O*-(tetrahydropyran-2-yl)-β-D-xylofuranosyl]-8-bromoadenine (6). A solution of **5** (7.38 g, 11.42 mmol) and dry sodium acetate (1.89 g) in DMF (80 ml) was stirred at room temperature for 2 d, then evaporated to dryness. The residue was partitioned between benzene (200 ml) and water (100 ml) and the organic layer was washed with water (100 ml), dried over $MgSO_4$ and concentrated to a small volume. The residue was chromatographed over a column of silica gel 60 (4.0× 40 cm) with 33 - 83% AcOEt in benzene (4 l) to give a caramel (3.06g, 48%). UV λmax (MeOH) 264 nm, λmax (0.1N HCl) 263 nm, λmax (0.1N NaOH) 264 nm. MS *m/z*: 556, 558 (M^++1).

8-Bromo-9-[2,5-di-*O*-(tetrahydropyran-2-yl)-β-D-xylofuranosyl]adenine (7). In a manner similar to that described in the section of **4**, the acetate **6** (3.0 g, 5.4 mmol) was converted to the xyloside **7** with 1M NaOH (7.5 ml), then separated by silica gel column chromatography. Evaporation of the fraction gave a caramel, which was crystallized from EtOH to give white crystals (1.90 g, 69%). mp 168-170℃. UV λmax (MeOH) 264 nm, λmax (0.1N HCl) 264 nm, λmax (0.1N NaOH) 264 nm. MS *m/z*: 513, 515 (M^+), 428, 430 (M^+ - C_5H_9O). *Anal.* Calcd for $C_{20}H_{28}BrN_5O_6$: C, 46.70; H, 5.49; N, 13.62. Found: C, 46.35; H, 5.42; N, 13.37.

8-Bromo-3'-deoxy-3'-fluoro-2',5'-di-*O*-(tetrahydropyran-2-yl)adenosine (8). To a cooled solution (- 60℃) of **7** (1.28 g, 2.5 mmol)) in a mixture of CH_2Cl_2 (25 ml) and pyridine (2.5 ml, 12 eq) was added DAST (1.35 ml, 4 eq) dropwise under N_2 atmosphere. The solution was heated under reflux for 6 h, then cooled. The mixture was poured into the stirred solution of 5% $NaHCO_3$ (100 ml) and diluted with CH_2Cl_2 (50 ml). The organic layer was washed with water twice (100 ml), dried over $MgSO_4$ and evaporated to a small volume. The solution was chromatographed over a column of silica gel 60 (3.0×45 cm) with 0 - 20% AcOEt in hexane (2 l). From the first fraction **8** was obtained as a caramel (736 mg, 57%). UV λmax (MeOH) 263.5 nm.

8-Bromo-3'-deoxy-3'-fluoroadenosine (9). A solution of **8** (488 mg, 0.95 mmol)) in EtOH (20 ml) was stirred in the presence of pyridinium *p*-toluenesulfonate (240 mg, 1 eq) at 80℃ for 1 d and the solution was concentrated to 3 ml. This solution was chromatographed over a column of silica gel 60 (3.0×40 cm) with 2 - 15% EtOH in $CHCl_3$ (2 l) to give white crystals (180 mg, 55%). UV λmax (MeOH) 264 nm, λmax (0.1N HCl) 263 nm, λmax (0.1N NaOH) 265 nm. ^{1}H-NMR (DMSO-d_6) δ: 8.14 (1H, s, H2), 7.65 (2H, br s, NH_2), 5.97 (1H, d, J = 6.1 Hz, 2'-OH), 5.87 (1H, d, J = 8.1 Hz, H1'), 5.78 (1H, br s, 5'-OH), 5.37 (1H, m, H2'), 5.15 (1H, dd, J = 4.2, 54.4 Hz, H3'), 4.33 (1H, dt, J = 4.2, 27.3 Hz, H4'), 3.70 (1H, dd, J = 4.4, 12.2 Hz, H5'a), 3.63 (1H, m, H5'b). MS *m/z*: 347, 349 (M^+), 317, 319 (M^+-CH_2O), 256, 258 (B^++C_2H_3O), 242, 244 (B^++CHO), 213, 215 (B^+).

3'-Deoxy-3'-fluoroadenosine (10). To a suspension of **9** (81 mg, 0.23 mmol) in EtOH (50 ml) was added 5% Pd/C (20 mg) and the solution was stirred under H_2 atmosphere at 50℃ for 2 d, then cooled. The catalyst was filtered off and the filtrate was evaporated to give a residue, which was dissolved in a small amount of EtOH and chromatographed over a column of silica gel 60 (2.6×33 cm) with 10 - 25% EtOH in $CHCl_3$ (1 l). Evaporation of the first fraction gave **10**[6),7)] as white crystals (45 mg, 72%). UV λmax (MeOH) 259 nm, λmax (0.1N HCl) 258 nm, λmax (0.1N NaOH) 259 nm. ^{1}H-NMR (DMSO-d_6) δ: 8.37 (1H, s, H8), 8.16 (1H, s, H2), 7.43 (2H, br s, NH_2), 5.94 (1H, d, J = 8.3 Hz, H1'), 5.92 (1H, d, J = 6.3 Hz, 2'-OH), 5.74 (1H, br s, 5'-OH), 5.15 (1H, dd, J = 4.2, 54.4 Hz, H3'), 4.94 (1H, m, H2'), 4.30 (1H, dt, J = 3.7, 27.6 Hz, H4'), 3.67 (2H, m, H5').

3'-Deoxy-3'-fluoro-8-mercaptoadenosine (11). A solution of **9** (70 mg, 0.2 mmol) and thiourea (26 mg, 0.33 mmol) in EtOH (5 ml) was refluxed overnight, then cooled. The solution was evaporated to dryness and the residue was crystallized from 50% EtOH (1 ml) to give **11** as white crystals (37 mg, 61%). mp 169-174℃. UV λmax (MeOH) 309 nm, λmax (0.1N HCl) 310 nm, λmax (0.1N NaOH) 300-305 nm. ^{1}H-NMR (DMSO-d_6) δ: 12.59 (1H, s, N^7-H), 8.13 (1H, s, H2), 6.99 (2H, br s, NH_2), 6.39 (1H, d, J = 8.1 Hz, H1'), 6.0 - 5.7 (2'-OH, 5'-OH), 5.36 (1H, ddd, J = 4.3, 8.1, 25.5 Hz, H2'), 5.08 (1H, dd, J = 4.3, 54.4 Hz, H3'), 4.22 (1H, dt, J = 4.2, 27.0 Hz, H4'), 3.6 (2H, m, H5'a, H5'b). MS *m/z* 301(M^+). *Anal.* Calcd for $C_{10}H_{12}FN_5O_3S \cdot 0.5H_2O$: C, 38.71; H, 4.22; N, 22.57. Found: C, 38.81; H, 4.28; N, 22.34.

3'-Deoxy-3'-fluoro-8-oxyadenosine (12). A solution of **9** (70 mg, 0.2 mmol) and sodium acetate (300 mg, 3.66 mmol) in a mixture of acetic acid (2.5 ml) and acetic anhydride (1.4 ml) was refluxed overnight, then cooled. The solution was evaporated to dryness and the residue was dissolved in EtOH (20 ml), kept at room temperature for 1 d, and evaporated. The residue was dissolved in $CHCl_3$ (30 ml) and the organic layer was

washed with water (20 ml), dried over $MgSO_4$ and evaporated to give the triacetate as a gum. The product was treated with MeOH saturated with ammonia at 0℃ (5 ml) at room temperature for 1 d and concentrated to a small volume, which was chromatographed over a column of silica gel 60 (2.0×18 cm) with 10 - 33% EtOH in $CHCl_3$ (500 ml) to give **12** as white crystals (37.4 mg, 65%). mp 230-231℃. UV λmax (MeOH) 270 nm, λmax (0.1N HCl) 267, 285 nm (sh), λmax (0.1N NaOH) 282 nm. ^{1}H-NMR (DMSO-d_6) δ: 10.42 (1H, s, N^7-H), 8.01 (1H, s, H2), 6.59 (2H, br s, NH_2), 6.0 - 5.7 (2'-OH, 5'-OH), 5.69 (1H, d, J = 7.6 Hz, H1'), 5.16 (1H, ddd, J = 4.1, 7.6, 25.6 Hz, H2'), 5.03 (1H, dd, J = 4.1, 54.6 Hz, H3'), 4.18 (1H, dt, J = 4.4, 26.8 Hz, H4'), 3.57 (2H, m, H5'). MS *m/z* 284 (M^+-1). *Anal.* Calcd for $C_{10}H_{12}FN_5O_4 \cdot 0.8H_2O$: C, 40.08; H, 4.57; N, 23.37. Found: C,40.38; H,4.41; N,23.13.

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