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Improved Synthesis of 5′-Deoxy-5′-adenosineacetic Acid

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An improved synthesis of 5′-deoxy-5′-adenosineacetic acid (**5**), which is useful as a model nucleotide of adenosine-5′-monophosphate, is presented.

Keywords——5′-deoxy-5′-adenosineacetic acid; adenosine-5′-monophosphate; Pfitzner–Moffatt oxidation; benzyloxycarbonylmethylenetriphenylphosphorane; Wittig reaction; hydrogenolysis

5′-Deoxy-5′-adenosineacetic acid (AAA) (**5**), which was first synthesized by Follmann,[1] is a useful model nucleotide of adenosine-5′-monophosphate (AMP), because it has been shown that the acid AAA (**5**) can replace AMP in oligonucleotides and as a substrate for enzymes, such as AMP aminohydrolase.[2]

Recently, Ishida *et al.*[3] reported the determination of the conformation of **5** by X-ray crystal structure analysis: *anti* for the glycosidic bond, *exo*-C(3′) for the sugar puckering, *gauche-trans* for the orientation about the C(4′)–C(5′) bond, and the carboxy group is linked to the N(6) and N(7) atoms of the neighboring adenine ring by two hydrogen bonds. During the course of our synthetic work on amides and peptides containing the acid AAA (**5**), which will compete with aminoacyl AMP in the reaction of aminoacyl transfer ribonucleic acid (tRNA) synthesis, and on nucleotide-like oligomers containing a carboxy ester linkage instead of a phosphate group, we required a convenient source of **5**.

The outlines of the synthetic method reported by Follmann[1] are as follows: a *p*-anisylidene group is used for the protection of the 2′,3′-*cis*-diol group. Pfitzner–Moffatt oxidation of 2′,3′-*O*-*p*-anisylideneadenosine gives the 5′-carboxaldehyde, which is subsequently condensed with ethoxycarbonylmethylenetriphenylphosphorane to give the α,β-unsaturated ester. This is hydrolysed with 80% acetic acid to afford **6** in only 12.5% yield based on the *p*-anisylideneadenosine. Catalytic reduction of **6** in the presence of 5% palladium-barium sulfate, followed by hydrolysis with Dowex 1 X-2 (OH^-) gives **5**. In the present paper, we report an improved and convenient synthesis of **5** starting from 2′,3′-*O*-ethoxymethyleneadenosine (**2**),[4] which can be readily prepared from adenosine (**1**) in 94.4% yield.[5] After Pfitzner–Moffatt oxidation of **2** under the same conditions as reported by Follmann, 2′,3′-*O*-ethoxymethyleneadenosine-5′-carboxaldehyde (**3**), generated *in situ*, was treated with benzyloxycarbonylmethylenetriphenylphosphorane,[6] which was recently used for the synthesis of a 5′-deoxy-5′-thymidineacetic acid derivative,[7] for 36 h at 37°C. After removal of the protecting group with 80% acetic acid, 5′-dehydro-5′-benzyloxycarbonylmethyleneadenosine (**4**), mp 154—155°C, could be isolated in 34.6% yield by a simplified work-up, as described in Experimental, followed by chromatography on silica gel. The proton magnetic resonance (PMR) spectrum of **4** is consistent with the *trans* geometry of the double bond ($J_{5',6'}=16$ Hz) and with the *trans* relationship for the C(3′)- and C(4′)-protons ($J_{3',4'}=5$ Hz). Catalytic reduction of **4** over 10% palladium on charcoal (Pd-C) gave the desired carboxylic acid (**5**), mp 242—245°C (lit.,[1] mp 233—234°C), in a single step in 97% yield. Thus, the overall yield for the conversion of **1** into **5** has been increased from 6.45% (by Follmann) to 31.7%.

Analogously, compound **3**, generated *in situ*, was condensed with ethoxycarbonylmethylenetriphenylphosphorane followed by treatment with 80% acetic acid to give 5′-dehydro-5′-

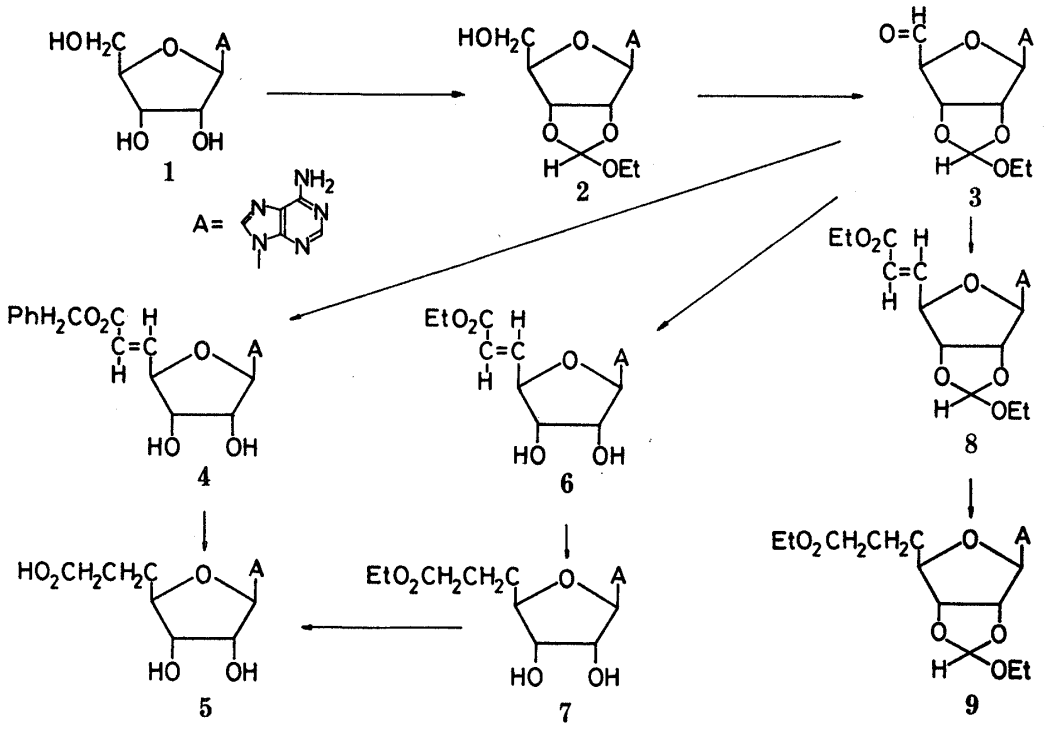

Chart 1

deoxy-5′-ethoxycarbonylmethyleneadenosine (**6**), mp 168—170° (lit.,[1] mp 178—183°C), in 32.2% yield; PMR data were identical with the reported values.[1] This nucleoside **6** was eventually derived to **5** *via* **7** according to the method of Follmann. Furthermore, 5′-dehydro-5′-deoxy-2′,3′-*O*-ethoxymethylene-5′-ethoxycarbonylmethyleneadenosine (**8**) was isolated in 53% yield as a hard glass which showed a single spot on thin-layer chromatography (TLC); its PMR spectrum was in accord with the reported structure. Hydrogenation of **8** in 0.5 M acetic acidsodium acetate (pH 5) buffer in ethanol in the presence of 10% Pd-C gave ethyl 5′-deoxy- 2′,3′-*O*-ethoxymethylene-5′-adenosineacetate (**9**) as a hard glass in 81.9% yield. Preparative layer chromatography (PLC) of the hard glassy products (**8** and **9**) successfully yielded pure crystalline materials. However, the stereochemistries of the ethoxymethylene group in compounds **8** and **9** remain undetermined. Alkaline hydrolysis of **9** readily gave 5′-deoxy-2′,3′-*O*-ethoxymethylene-5′-adenosineacetic acid [PMR(DMSO-d_6) δ: 6.10 (1H, s, C<u>H</u>–OEt)] as a hard glass. This compound should be useful as a building unit for the synthesis of nucleotide-like oligomers containing 5′-deoxy-5′-adenosineacetic acid.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO model IRA-1 spectrophotometer and the ultraviolet (UV) spectra on a JASCO UVIDEC-505 spectrophotometer. The PMR spectra were obtained at 90 MHz with a Hitachi R-40 and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal standard. TLC was performed with Kieselgel $60F_{254}$ (Merck) with $CHCl_3$–MeOH (5: 1 or 9: 1) as an eluent. PLC was carried out on plates (20×20 cm, 0.75 mm thick) coated with Kieselgel PF_{254} (Merck). For silica gel column chromatography, Kieselgel 60 (Merck) was used unless otherwise noted.

5′-Dehydro-5′-deoxy-5′-benzyloxycarbonylmethyleneadenosine (**4**)——A stirred mixture of 2′,3′-*O*-ethoxymethyleneadenosine (**2**) (3.23 g, 10 mmol), dry pyridine (0.8 ml, 10 mmol) and trifluoroacetic acid (0.38 ml, 5 mmol) in dry dimethyl sulfoxide (DMSO) (50 ml) was treated with *N*,*N*′-dicyclohexylcarbodiimide (DCC) (6.72 g, 30 mmol). The whole mixture was stirred at room temperature for 2 d, then benzyloxycarbonylmethylenetriphenylphosphorane (5.0 g, 15 mmol) was added. The reaction mixture was stirred for an additional 24 h at 37°C, then diluted with AcOEt (200 ml). Oxalic acid (3.6 g, 40 mmol) was added

to decompose excess DCC under ice-cooling. The resulting *N*,*N*′-dicyclohexylurea was removed by filtration, and the filtrate was diluted with additional AcOEt (300 ml), then washed with saturated $NaHCO_3$ solution (250 ml × 2). The aqueous solution was reextracted with AcOEt (150 ml), and the combined organic layer was evaporated to dryness *in vacuo*. The residue was dissolved in 80% AcOH (100 ml), and the acidic solution was allowed to stand for 24 h at 37°C. After removal of the solvent by evaporation, the residue was dissolved in AcOEt (300 ml) and the solution was washed with saturated $NaHCO_3$ solution (50 ml). Drying ($MgSO_4$) followed by evaporation gave an oil, which was subjected to silica gel (100 g) column chromatography. The fractions eluted with benzene–MeOH (10: 1) were combined and concentrated to give a solid, which was recrystallized from AcOEt to give **4** as colorless needles of mp 154—155°C. The residue obtained by concentration of the filtrate was rechromatographed on silica gel (70 g). The fractions eluted with AcOEt–MeOH (5: 1) were combined and concentrated to give a solid, which was recrystallized from AcOEt to give additional **4**. Total yield, 1.375 g (34.6%). IR ν_{max}^{KBr} cm^{-1}: 1710(CO). UV λ_{max}^{EtOH} nm (ε): 259 (1.49×10^4). PMR (DMSO-d_6) δ: 4.32 [1H, dd, $J_{3',4'}=5$ Hz, C(3′)–H], 4.61 [1H, ddd, $J_{4',5'}=7$ Hz, $J_{4',6'}=1$ Hz, C(4′)–H], 4.80 [1H, t, $J_{2',3'}=4.5$ Hz, C(2′)–H], 5.17 (2H, s, CH_2Ar), 5.97 [1H, d, $J_{1',2'}=5$ Hz, C(1′)–H], 6.10 [1H, dd, $J_{5',6'}=16$ Hz, C(6′)–H], 7.11 [1H, dd, C(5′)–H], 7.34 (5H, s, Ar–H), 8.10 [1H, s, C(2)–H], 8.34 [1H, s, C(8)–H]. *Anal.* Calcd for $C_{19}H_{19}N_5O_5$: C, 57.42; H, 4.82; N, 17.63. Found: C, 57.14; H, 5.11; N, 17.82.

5′-Deoxy-5′-adenosineacetic Acid (5)——A suspension of **4** (397 mg, 1 mmol) and 10% Pd-C (400 mg) in 95% EtOH–AcOH (9: 1) (20 ml) was shaken at 50°C in a Skita apparatus under 4 kg/cm² pressure of H_2 for 10 h. The reaction mixture was filtered through celite and the insoluble material with celite was extracted with hot H_2O (50 ml × 2). The filtrate and extracts were combined and evaporated to dryness *in vacuo* to give **5** (302 mg, 97.6%), whose IR and PMR spectra were identical with those of an authentic sample, prepared from **6** according to the method of Follmann. Recrystallization from H_2O afforded an analytical sample of **5**, mp 242—243°C (lit.,[1] 233—234°C). *Anal.* Calcd for $C_{12}H_{15}N_5O_5$: C, 46.00; H, 4.85; N, 22.66. Found: C, 46.52; H, 4.58; N, 22.58.

5′-Dehydro-5′-deoxy-5′-ethoxycarbonylmethyleneadenosine (6)——A solution of 2′,3′-*O*-ethoxymethyleneadenosine-5′-carboxaldehyde (**3**), generated *in situ* from **2** (9.7 g, 30 mmol) as described above, in DMSO (150 ml) was treated with ethoxycarbonylmethylenetriphenylphosphorane (15 g, 43.5 mmol). Work-up, separation, and crystallization as described for **4** gave **6**, mp 168—170°C (lit.,[1] mp 178—183°C), whose PMR spectral data were identical with the reported values. *Anal.* Calcd for $C_{14}H_{17}N_5O_5$: C, 50.14; H, 5.11; N, 20.89. Found: C, 50.22; H, 5.23; N, 21.09.

5′-Dehydro-5′-deoxy-5-ethoxycarbonylmethylene-2′,3′-*O*-ethoxymethyleneadenosine (8)——DCC (12.5 g, 60 mmol) was added to a stirred mixture of **2** (6.46 g, 20 mmol), dry pyridine (1.6 ml, 20 mmol), and trifluoroacetic acid (0.72 ml, 10 mmol) in dry DMSO (100 ml). After the mixture had been stirred for 24 h at room temperature, ethoxycarbonylmethylenetriphenylphosphorane (10.0 g, 29 mmol) was added. The mixture was stirred for another 3 d at room temperature, then diluted with AcOEt (400 ml). Oxalic acid (7.2 g, 80 mmol) was added and the resulting *N*,*N*′-dicyclohexylurea was removed by filtration. The filtrate was washed with saturated $NaHCO_3$ solution (250 ml × 2), and the aqueous layer was reextracted with AcOEt (150 ml). The combined organic layers were dried over $MgSO_4$, then concentrated *in vacuo*, and the residue was applied to a short column (6.5 × 10 cm) of Silica gel HF_{254} (Merck). The fractions containing only the new nucleoside (eluted with benzene–MeOH (25: 1)) were combined, and concentrated to dryness to give **8** as a hard glass. The fractions contaminated by impurities were concentrated, and the residue was rechromatographed on Lobar column (LiChroprep Si 60 Größe C) with benzene–MeOH (20: 1). Concentration of the desired fractions *in vacuo* gave additional **8** as a hard glass. Total yield, 4.0 g (51.1%). This product was sufficiently pure for the next reaction. Purification by PLC with $CHCl_3$–MeOH (9: 1) gave a solid, which was recrystallized from AcOEt to give an analytical sample of **8**, mp 114—119°C. IR ν_{max}^{KBr} cm^{-1}: 1715 (CO). UV λ_{max}^{EtOH} nm (ε): 259.5 (1.44×10^4). PMR (DMSO-d_6) δ: 1.19 and 1.24 (each 3H, each t, $J=7$ Hz, $CO_2CH_2\underline{CH_3}$ and $OCH_2\underline{CH_3}$), 3.70 and 4.10 (each 2H, each q, $J=7$ Hz, $OC\underline{H_2}CH_3$ and $CO_2C\underline{H_2}CH_3$), 4.93 [1H, m, C(4′)–H], 5.15 [1H, dd, $J_{3',4'}=2.5$ Hz, C(3′)–H], 5.58 [1H, dd, $J_{2',3'}=6$ Hz, C(2′)–H], 5.77 [1H, dd, $J_{6',4'}=1$ Hz, C(6′)–H], 6.24 (1H, s, CH), 6.36 [1H, d, $J_{1',2'}=2$ Hz, C(1′)–H], 6.95 [1H, dd, $J_{5',4'}=5.5$ Hz, $J_{5',6'}=14.5$ Hz, C(5′)–H], 8.14 [1H, s, C(2)–H], 8.37 [1H, s, C(8)–H]. *Anal.* Calcd for $C_{17}H_{21}N_5O_6$: C, 52.17; H, 5.41; N, 17.90. Found: C, 52.14; H, 5.70; N, 17.88.

Ethyl 5′-Deoxy-2′,3′-*O*-ethoxymethylene-5′-adenosineacetate (9)——A suspension of **8** (2.55 g, 6.5 mmol), 10% Pd-C (1.28 g), and 0.5 M AcOH–AcONa buffer (pH 5) (10 ml) in EtOH (30 ml) was shaken in a Skita apparatus under 4 kg/cm² pressure of H_2 for 15 h at room temperature. The reaction mixture was filtered through celite, and the filtrate was evaporated to dryness. AcOEt (50 ml) and H_2O (25 ml) were added to the residue. The organic layer was separated, washed with H_2O and dried over $MgSO_4$. Removal of the solvent *in vacuo* gave **9** (2.10 g, 81.9%) as a hard glass which showed a single spot on TLC. Purification by PLC with $CHCl_3$–MeOH (9: 1) gave a solid, which was recrystallized from AcOEt to give an analytical sample of **9**, mp 138—139°C. IR ν_{max}^{KBr} cm^{-1}: 1734 (CO). UV λ_{max}^{EtOH} nm (ε): 259.3 (1.53×10^4). PMR (DMSO-d_6) δ: 1.15 and 1.24 (each 3H, each t, $J=7$ Hz, $CO_2CH_2\underline{CH_3}$ and $OCH_2\underline{CH_3}$), 1.99 [2H, q, $J_{5',4'}=J_{5',6'}=7$ Hz, C(5′)–H], 2.42 [2H, t, $J=7$ Hz, C(6′)–H], 3.69 and 4.06 (each 2H, each q, $J=7$ Hz, $OC\underline{H_2}CH_3$ and $CO_2C\underline{H_2}$-

CH_3), 4.28 [1H, m, C(4′)–H], 4.94 [1H, dd, $J_{4',3'}=4$ Hz, C(3′)–H], 5.55 [1H, dd, $J_{2',3'}=7$ Hz, C(2′)–H], 6.20 (1H, s, CH), 6.27 [1H, d, $J_{1',2'}=3$ Hz, C(1′)–H], 8.26 [1H, s, C(2)–H], 8.49 [1H, s, C(8)–H]. *Anal.* Calcd for $C_{17}H_{23}N_5O_6$: C, 51.90; H, 5.89; N, 17.80. Found: C, 52.09; H, 5.99; N, 17.70.

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References and Notes

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