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STUDIES OF NUCLEOSIDES AND NUCLEOTIDES—LXV[1]

PURINE CYCLONUCLEOSIDES-26 A VERSATILE METHOD FOR THE SYNTHESIS OF PURINE O-CYCLO-NUCLEOSIDES. THE FIRST SYNTHESIS OF 8,2'-ANHYDRO-8-OXY 9-β-D-ARABINOFURANOSYLGUANINE

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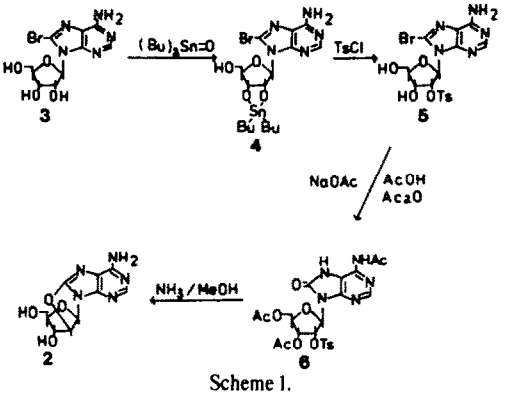
Abstract—8-Bromoadenosine and 8-bromoguanosine were tosylated on 2'-hydroxyls via 2', 3'-O-dibutylstannylene compounds in yields of 79 and 54%, respectively. 2'-O-Tosyl-8-bromoadenosine was converted to the 8-oxy derivative by treatment with sodium acetate in acetic acid-acetic anhydride mixture and cyclized by heating with ammonia-methanol to afford 8,2'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine. 2'-O-Tosylguanosine was analogously converted to the 8-oxy compound and cyclized by heating with sodium acetate in DMF to 8,2'-anhydro-8-oxy-9-β-D-arabinofuranosylguanine, which was the first example of the unprotected 8,2'-O-cycloguanosine.

The knowledge of purine 8-cyclonucleosides has increased[2,3] but the selective introduction of leaving groups such as methyl- or toluenesulfonyl to the 2'- or 3'-hydroxyl of nucleosides has proved difficult.[4] Ikehara and Uesugi[5] have sucessfully introduced a tosyl group to the 2'-OH of adenosine 5'-phosphate. Mian *et al.*[6] recently reported the 2'-tosylation of 8-bromoadenosine or guanosine 3',5'-cyclic phosphate. Although the synthesis of 8,2' - S - cyclonucleosides has been achieved by treating 8-mercapto derivatives with diphenyl carbonate[7-10] or trimethylsilyl chloride,[11] 8,2' - O - cyclonucleosides could not be obtained in good yields. Especially in the case of guanosine, only a 3'-O-mesyl derivative of 8,2'-O-cyclonucleoside **1a** has been synthesized.[12] Recently, Wagner *et al.*[13] reported a method for the selective introduction of a tosyl group to 2'-OH of nucleosides using di-n-butyltin oxide as the activating reagent. Utilysing this reagent we now report a versatile method for the synthesis of 8,2' - anhydro - 8 - oxy - 9 - β - D-arabinofuranosyladenine **2** and 8,2' - anhydro - 8 - oxy - 9 - β - D-arabinofuranosylguanine **1b**. The latter compound was the first example of a guanine 8,2'-O-cyclonucleoside and may be useful for various reactions, which have been developed for adenine cyclonucleosides.

When 8-bromoadenosine[4] **3** was heated with an equivalent amount of di-O-n-butyltin oxide,[14] a colourless crystalline material, m.p. 220–221°, was obtained in a yield of 92%. Elemental analysis and UV absorption at 266 nm suggested that this compound was 8 - bromo - 2',3' - O - dibutylstannyleneadenosine **4**. The mass spectrum of **4** gave only fragment ions corresponding to 8-bromoadenine.[15] Compound **4** reacted with triethylamine and tosyl chloride to give 2' - O - tosyl - 8 - bromoadenosine **5** identified as follows. Elemental analysis and UV absorption spectra suggested that this compound has a tosyl group. The NMR spectrum taken in d_6-DMSO showed signals of a Me group and protons of a tosyl group. A signal of H-2' appeared at 5·72, showing that this carbon must be substituted. The IR band at 1152 cm^{-1} showed the presence of a covalent tosylate. UV absorption spectra also supported the introduction of a tosyl group to the carbohydrate moiety.



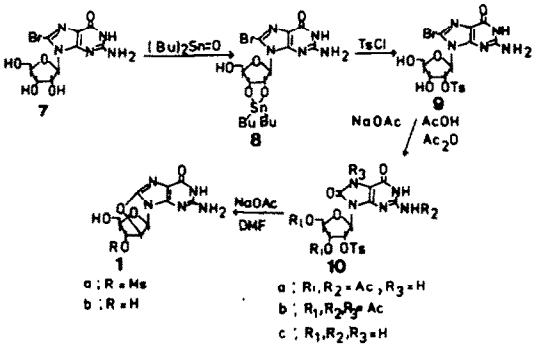
Scheme 1.

Compound **5** on heating in a mixture of acetic anhydride-glacial acetic acid containing excess sodium acetate gave 2' - O - tosyl - N^6,3',5' - triacetyl - 8 - oxyadenosine **6**, m.p. 202–204°. UV absorption spectrum of this compound showed maxima at 290–306 nm suggesting the structure to be correct. NMR spectra of compound **6** showed signals of N^6-H and N^7-H at 9·51 and 9·17δ. Signals belonging to the carbohydrate moiety (4·34–6·09 δ) were also shifted relative to 8 - oxy - adenosine towards low field. IR bands at 1720 cm^{-1} showed a C=O group at the 8-position.

Compound **6** was cyclized to 8,2' - anhydro - 8 - oxy - 9 - β - D - arabinofuranosyladenine (8,2' - O - cycloadenosine) **2** in a yield of 64%. This material was identical with an authentic sample[16] in mixed m.p., paper chromatography in two solvent systems and UV absorption properties. This method for the synthesis of 8,2'-O-cycloadenosine is convenient for the handling of large scale material.

Synthesis of 8,2' - anhydro - 9 - β - D - arabinofuranosylguanine **1b** was then attempted by this method. 8-Bromoguanosine **7** and one equivalent of di-n-butyltin oxide afforded 8 - bromo - 2',3' - dibutylstannyleneguanosine **8**, m.p. 237–239°, in a yield of 75%. UV absorption maximum at 262 nm and mass fragment peaks of *m/e* 229 and 231 corresponding to bromoguanine

ion showed the structure to be correct. The 2' - O - tosyl - 8 - bromoguanosine **9** was prepared in a yield of 63% by treatment of crude dibutylstannylene compound **8** with triethylamine and tosyl chloride. UV absorption maxima at 232 and 264 nm showed the introduction of a tosyl group to the carbohydrate moiety. The IR band at 1160 cm^{-1} suggested the presence of a tosyl group. The NMR spectra showed signals assigned to H-2' (5·79), which appeared at lower field than that of H-1' (5·81), a methyl signal and protons of a tosyl group.



Scheme 2.

Compound **9** when treated with sodium acetate in acetic anhydride-acetic acid mixture yielded the 8-oxy compounds **10a** and **b** identified as N^2,3',5' - tri - acetyl and N^2,N^7 - 3',5' - tetraacetyl derivatives, respectively, by the absorption spectra and the migrating ratio in TLC. When these compounds were treated with methanolic ammonia 2' - O - tosyl - 8 - oxyguanosine **10c** was obtained, m.p. 185–188°, and the structure of **10c** was confirmed by UV and IR spectra. The 2' - tosyl - 8 - oxyguanosine **10c** was converted to 8,2' - anhydro - 8 - oxy - 9 - β - D - arabinofuranosylguanine **1b** in a yield of 45%. The structure of **1b** was confirmed by the following criteria. UV absorption maxima at 247 and 287 nm closely resembled those found in 3'-mesyl derivative.[12] The NMR spectra in d_6-DMSO showed signals of H-1' at 6·35 δ and H-2' at 5·56 having a coupling constant $J_{1'2'} = 5·0$ Hz which agrees with the previous observations on 8,2' - cyclonucleosides.[7] CD spectra taken in acid, alkali and neutral solution are shown in Fig. 1. As has been observed in the case of other purine cyclonucleosides,[17] bands in the B-region showed a positive Cotton effect. This fact strongly suggested that compound **1b** has the cyclonucleoside structure. A comparison of this result with the CD spectra of guanosine suggested that in neutral solution guanosine had a negative band corresponding to the *anti* conformation and in acid it changed to positive, because of the change of the conformation to *syn*.[18] This argument is not consistent, because transition moments of the base could be changed by the protonation probably at the N^7-position. The present compound **1b** had the same sign of the Cotton band in the B-region both in neutral and acid media. Since the base moiety of this compound is rigidly fixed at the angle ($\phi_{CN} = -120°$),[19] it could not change the conformation even by protonation. Therefore, it may be stated that the *syn-anti* conformation change in guanosine can be interpreted by the change in the sign of the Cotton effect.

The present method of O-cyclonucleoside synthesis seems to be useful for the preparation of variously substituted purine nucleosides. Work along these lines is now in progress and will be reported in subsequent papers.

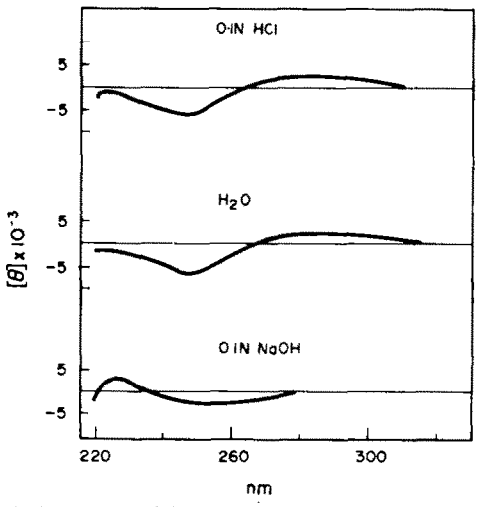


Fig. 1. CD spectra of 8,2' - anhydro - 8 - oxy - 9 - β - D - arabinofuranosylguanine.

EXPERIMENTAL[20]

2',3' - O - Di - n - butylstannylene - 8 - bromoadenosine **4**. 8-Bromoadenosine[4] (4·152 g, 12 mmol) and di-n-butyltin oxide (3·0 g, 12 mmol) were dissolved in MeOH (300 ml) and the mixture refluxed for 5·5 h. After cooling, the colourless crystalline ppts were collected, yield 6·28 g (11 mmol, 92%), m.p. 220–221° (Found: C, 37·02; H, 4·72; N, 12·08; Br, 14·00. Calc for $C_{18}H_{28}N_5O_4BrSn$: C, 37·47; H, 4·89; N, 12·14; Br, 13·85.); UV: λ_{max}^{EtOH} 266 nm (ε 16,100); mass spectrum: *m/e* 213 (bromoadenine − H), 215 (bromoadenine + H), 331–339 (dibutylstannyleneribose-CH_2OH).

2' - O - Tosyl - 8 - bromoadenosine **5**. Compound **4** (3·82 g, 6·7 mmol) was dissolved in MeOH (170 ml) and triethylamine (14 ml, 15 equiv) and tosyl chloride (19·0 g, 15 equiv) were added. The mixture was stirred at room temp for 2 h yielding 2·81 g (5·6 mmol, 86%) which on recrystallisation had m.p. 195° (dec 220°) (Found: C, 41·10; H, 4·36; N, 14·30; S, 6·33; Br, 16·30. Calc for $C_{17}H_{18}N_5O_6$ SBr: C, 40·81; H, 3·63; N, 14·00; S, 6·41; Br, 15·97); UV: $\lambda_{max}^{50\%EtOH}$ nm (ε) 267 (14,000), 230 (shoulder, 13100); $\lambda_{max}^{0·1NHCl}$ 264·5 (16,100), 230 (shoulder, 13,000); $\lambda_{max}^{0·1NNaOH}$ 267 (14,000); IR ν_{max}^{KBr} 1152 cm^{-1} (covalent tosylate); NMR: (d_6-DMSO) 7·95 (s, 1H, H-2), 7·54 (b, 2H, 6-NH_2), 7·37 (d, 2H, tosyl-Ha), 6·98 (d, 2H, tosyl-Hb), 6·08 (d, 1H, OH-3'), 5·97 (d, 1H, H-1'), 5·72 (q, 1H, H-2'), 5·59 (t, 1H, OH-5'), 4·41 (h, 1H, H-3'), 4·10 (m, 1H, H-4'), 3·64 (m, 2H, H-5'), 2·27 (s, 3H, tosyl-CH_3), $J_{H1'-H2'}$ = 7·0 Hz, $J_{H2'-H3'}$ = 4·0 Hz, $J_{H3'-H4'}$ = 1·5 Hz, $J_{H3'-OH3'}$ = 5·0 Hz, $J_{H5'-OH5'}$ = 4·0 Hz. R_f's in paper chromatography are listed in Table 1.

8 - Oxy - 2' - O - tosyl - N^6,3',4' - triacetyladenosine **6**. 8 - Bromo - 2' - tosyladenosine (1·0 g, 2 mmol) was dissolved in a mixture of AcOH (25 ml) and Ac_2O (25 ml) containing anhyd NaOAc (3·0 g). The soln was heated under reflux for 3 h. The solvent was evaporated *in vacuo* and traces of Ac_2O were decomposed and removed by evaporation several times with EtOH (50 ml). The residue was suspended in $CHCl_3$ and water (70 ml, each), $CHCl_3$-layer was washed twice with sat aq $NaHCO_3$ (30 ml), and finally with water (30 ml × 2). Dried ($MgSO_4$) $CHCl_3$ soln was evaporated to give a glass (1·131 g). Recrystallisation of the residue from EtOH gave m.p. 202–204°. (Found: C, 49·09; H, 4·56; N, 12·46; S, 5·55. Calc for $C_{23}H_{27}N_5O_{10}S$: C, 49·02; H, 4·47; N, 12·43; S, 5·69); UV: $\lambda_{max}^{50\%EtOH}$ nm (ε) 235 (sh 14,900), 290 (12,900); $\lambda_{max}^{0·1NHCl}$ 275 (8,300), 306 (13,700); IR: ν_{max}^{KBr} 1160 cm^{-1} (covalent tosylate), 1720 cm^{-1} (8-C=O); NMR: ($CDCl_3$,) 9·51 (b, 1H, N^7-H), 9·17 (b, 1H, N^6-H), 8·32 (s, 1H, H-2), 7·70 (d, 2H, tosyl-Ha), 7·19 (d, 2H, tosyl-Hb), 6·21 (d, 1H, 1-1'), 6·09 (q, 1H, H-2') 5·67 (m, 1H, H-3'), 4·34 (m, 3H, H-4' and 5'), 2·36 (s, 3H, NAc-CH_3), 2·30 (s, 3H, tosyl-CH_3), 2·05 (s, 6H, OAc-CH_3). R_f's in paper chromatography were listed in Table 1.

8,2' - Anhydro - 8 - oxy - 9 - β - D - arabinofuranosyladenine **2**. The 8-oxy compound **6** obtained as a glass (852 mg) was

Table 1. R_f's in paper chromatography of synthesized compounds

Compound	Solvent A*	B
Adenosine	0·46	0·57
8-Br-2'-Ts-adenosine	0·79	0·77
8,2'-O-cyclo-adenosine	0·46	0·53
Guanosine	0·27	0·38
8-Br-2'-Ts-guanosine	0·79	0·77
8-OH-2'-Ts-guanosine	0·68	0·68
8,2'-O-cyclo-guanosine	0·25	0·37

*Solvent A, n-Butanol-acetic acid-water, 7:1:2; B, iso-propanol-conc. Ammonia-water, 7:1:2.

dissolved in MeOH (30 ml), through which ammonia gas was bubbled for 30 min under cooling at −20°. The mixture was sealed in a steel tube and heated at 60° for 7 h. Crystalline material appearing at the bottom of the tube was recrystallised from MeOH to give 245 mg (9·5 mmol, 64%) of **2**. TLC of the mother liquor showed that *ca.* 30% of the starting material **6** remained unchanged. This sample was identical with an authentic specimen of 8,2' - O - cycloadenosine[16] by the crieteria of paper chromatography (Table 1) and UV absorption properties.

8 - *Bromo* - 2',3', - O - *dibutylstannyleneguanosine* **8**. 8 - Bromoquanosine[21] (1·448 g, 4 mmol) and di-n-butyltin oxide (1·0 g, 4 mmol) in methanol (100 ml) were heated under reflux for 1 h. The ppt was removed by filtration and MeOH was evaporated *in vacuo*. The residue was recrystallised from ethanol-acetone mixture to give colourless crystals (1·77 g, 3·01 mmol, 75%), m.p. 237–239° (dec). (Found: C, 36·40; H, 4·96; N, 11·88, Br, 13·20. Calc for $C_{18}H_{28}N_5O_5BrSn$: C, 36·46; H, 4·76; N, 11·81; Br. 13·47); UV: λ_{max}^{EtOH} 262 nm (ϵ 16,200), 280 (sh, 11600); mass spectrum: *m/e* 229, 231 (bromoguanine); 331–339 (di-butylstannyleneribose − CH_2OH).

2' - O - *Tosyl* - 8 - *bromoguanosine* **9**. 8-Bromoguanosine (283 mg, 1 mmol) and dibutyltin oxide (250 mg, 1 mmol) in methanol (25 ml) was heated under reflux for 1 h. After cooling triethylamine (2·1 ml, 15 mmol) and tosyl chloride (15 mmol) were added successively. After keeping the mixture at room temp for 10 min, the solvent was evaporated. To the residue water (30 ml) was added and the soln was extracted with ether (30 ml). The aqueous layer was separated, washed again with ether (30 ml × 2) and kept in a freezer over-night, yielding colourless crystals (278 mg, 54%), m.p. 228° (dec). (Found: C, 39·46; H, 3·49; N, 13·56; S, 6·49; Br, 15·31. Calc for $C_{17}H_{18}N_5O_7BrS$: C, 39·55; H, 3·51; N, 13·56; S, 6·21; Br, 15·48); TLC: ($CHCl_3$–EtOH, 3:1) R_f 0·72 (8-bromoguanosine 0·20); UV: $\lambda_{max}^{50\%EtOH}$ 232, 264 nm; $\lambda_{max}^{0.1NHCl}$ 232, 263 nm; $\lambda_{max}^{0.1NNaOH}$ 269 nm; IR: ν_{max}^{KBr} 1160 cm^{-1} (covalent tosylate); NMR: (δ) 10·73 (br, 1H, N^1-H), 7·47 (d, 2H, Ts-Ha), 7·11 (d, 2H, Ts-Hb), 6·46 (br, 2H, 2-NH_2), 5·81, 5·79 (m, 2H, H-1' and H-2'), 4·84 (br, 2H, 3'- and 5'-OH), 4·37 (q, 1H, H-3'), 4·00 (m, 1H, H-4'), 3·61 (m, 2H, H-5'), 2·30 (s, 3H, Ts-CH_3). R_f's in paper chromatography were listed in Table 1.

When 8-bromoguanosine (1·448 g, 4 mmol) was used, 8 - bromo - 2' - O - tosylguanosine (1·308 g, 2·53 mmol) was obtained.

8,2' - *Anhydro* - 8 - *oxy* - 9 - *β* - D - *arabinofuranosylguanine* **1b**. 8 - Bromo - 2' - tosylguanosine (1·548 g, 3·0 mmol) was dissolved in a mixture of Ac_2O–AcOH (each 38 ml) containing anhyd NaOAc (4·5 g). The mixture was heated under reflux for 4 h. TLC ($CHCl_3$–EtOH, 20:1) at this stage showed two spots having R_f 0·33 (a) and 0·21 (b). Uv absorption properties of these compounds are listed in Table 2. Structures of N^2, N^7 - 3',5' - tetraacetyl- and N^2,3',5' - triacetyl - 2' - tosyl - 8 - oxyguanosine were assigned to compound (a) and (b), respectively. The solvent was evaporated *in vacuo* and the residue was evaporated with EtOH (30 ml × 3) to remove traces of Ac_2O. The residue was dispersed in $CHCl_3$ (150 ml) and water (150 ml) and $CHCl_3$ layer was separated. The $CHCl_3$ layer was washed with sat $NaHCO_3$ aq (50 ml × 2), water (50 ml × 2) and finally dried ($MgSO_4$). TLC of this solution showed only one spot of (b). $CHCl_3$ was evaporated and the residual glass dissolved in MeOH (30 ml), and ammonia was bubbled through for 30 min at −20°. After keeping the soln at room temp for 20 h, the solvent was evaporated. A portion of the residual glass was recrystallised from MeOH–EtOH-water mixture to give 8 - oxy - 2' - tosylguanosine, m.p. 185–188° (dec 200°) (Found: C, 44·62; H, 4·15; N, 15·32; S, 6·80. Calc for $C_{17}H_{19}N_5O_8S$: C, 45·03; H, 4·19; N, 15·45; S, 7·06); IR: ν_{max}^{KBr} 1160 cm^{-1} (covalent tosylate), 1720 cm^{-1} (8-C=O).

The glass obtained above was dissolved in DMF (200 ml) and heated with NaOAc (6 g) at 100° for 4 h with stirring. The mixture was cooled to 0°, ppt was removed by filtration, and concentrated to *ca.* 15 ml. The ppt was removed again and the solvent was evaporated carefully. The residual glass was recrystallised from EtOH-water. Amorphous aggregates were collected by centrifugation, washed with ether, and dried. The solid material (393 mg) thus obtained was recrystallised from water to give 8,2' - O - cycloguanosine (376 mg, 1·34 mmol, 45%). This material colourized from 250°, but did not melt at 265° (Found: C, 42·87; H, 3·98; N, 24·90. Calc for $C_{10}H_{11}N_5O_5$: C, 42·71; H, 3·94; N, 24·90); UV: $\lambda_{max}^{0.1NHCl}$ nm (ϵ) 246 (13,000), 285 (sh, 9,100); $\lambda_{max}^{H_2O}$ 247 (13,000), 287 (8,900); $\lambda_{max}^{0.1NNaOH}$ 250 (11,700), 270 (sh, 9,100); NMR: (d_6-DMSO, δ) 10·68 (br, 1H, N^1-H), 6·45 (br, 2H, 6-NH_2), 6·35 (d, 1H, H-1', $J_{1'-2'}$ = 5·0 Hz), 5·86 (d, 1H, 3'-OH, $J_{H3'-OH3'}$ = 4·0 Hz), 5·56 (q, 1H, H-2', $J_{2',3'}$ = 1·0 Hz), 4·91 (t, 1H, 5'-OH, $J_{H5'-OH5'}$ = 6·0 Hz), 4·47 (m, 1H, H-3'), 4·17 (hex, 1H, H-4'). R_f's in paper chromatography were listed in Table 1.

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Table 2. UV absorption properties of acetylated 2' - tosyl - 8 - oxy - guanosine

Compound	50% ethanol (nm)	H^+(nm)	OH^-(nm)
N^2,N^7,3',5'-tetraacetyl	230 (shoulder), 264, 298	230 (shoulder), 264, 298	276, 330 (shoulder)
N^2,3',5'-triacetyl	230 (shoulder), 265, 303	230 (shoulder) 265, 303	275, 330 (shoulder)

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