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STUDIES OF NUCLEOSIDES AND NUCLEOTIDES—XLI[1]

PURINE CYCLONUCLEOSIDES—8 SELECTIVE SULFONYLATION OF 8-BROMOADENOSINE DERIVATIVES AND AN ALTERNATE SYNTHESIS OF 8,2′- AND 8,3′-S-CYCLONUCLEOSIDES

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Abstract—5′-O-Trityl-, 5′-O-benzoyl-, 5′-O-acetyl- and unprotected 8-bromoadenosine were subjected to sulfonylation using sodium hydride and triisopropyl-benzenesulfonyl chloride in N,N-dimethylformamide solution. In each case 2′- and 3′-monosulfonylated compounds were the main products. The ratio of 2′- to 3′-sulfonylated compound increased as the bulkiness of 5′-substituted group increased. Each sulfonylated product was confirmed as to their structure by elemental analysis and UV and IR absorption properties as well as transformation to 8,2′- and 8,3′-S-cyclonucleosides. The present series of reactions affords a convenient and novel method for the synthesis of purine cyclonucleosides.

IN RECENT years the synthesis and properties of a variety of purine 8-cyclonucleosides have been reported.[2] These cyclonucleosides are useful for transformation of ribonucleosides to deoxyribonucleosides.[3–5] Especially, 8,2′-S- and 8,3′-S-cyclonucleosides of adenosine have afforded naturally occurring 2′-deoxy- and 3′-deoxyadenosine (the antibiotic cordycepin).[3, 6] However, tosylation of 5′-acetyl-8-bromoadenosine, with the intention of obtaining a suitable intermediate of cyclonucleosides, gave 2′- and 3′-tosylate in almost 1:1 ratio and made it difficult to separate the desired isomer for further reactions. In this respect, we attempted to control the attack of a sulfonylating reagent either on 2′- or 3′-OH group by the introduction of a bulky group in the 5′-position[7] and by the use of a bulky sulfonyl chloride as reagent. As the sulfonyl chloride we chose 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl),[8] because this reagent proved to be bulky enough so that it could not react with a nucleoside OH group in pyridine at room temperature.[9] Therefore, if a sugar OH group of various 5′-substituted adenosine derivatives could react with TPS-Cl, we should obtain information on the selectivity of sulfonylation for either the 2′- or 3′-OH group.

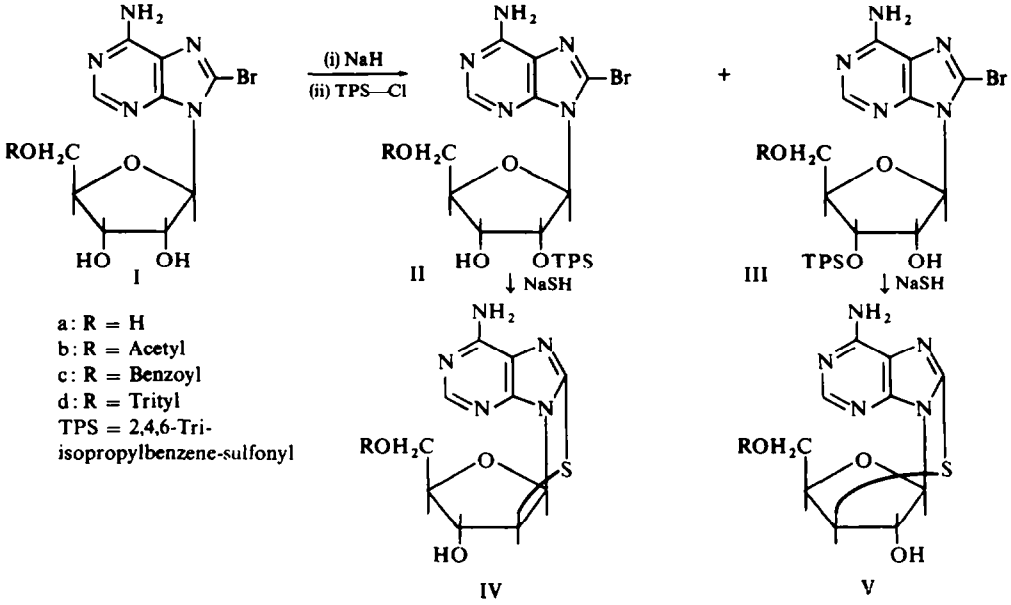
It was shown previously that nucleoside OH groups will dissociate to $-O^-$ by using sodium hydride in an aprotic dipolar solvent.[10, 11] We utilized this method for the reaction of 8-bromoadenosine derivatives with TPS-Cl. If a sulfonyl group is introduced into 8-bromoadenosine, this compound could be easily cyclized to yield S-cyclonucleosides, whose structures have been firmly established.[3] 8-Bromoadenosine[12] (Ia), 5′-O-acetyl-8-bromoadenosine[3] (Ib), 5′-O-benzoyl-8-bromoadenosine (Ic) and 5′-O-trityl-8-bromoadenosine (Id) were sulfonylated. Compound Ic was synthesized by benzoylation of 2′,3′-O-isopropylidene-8-bromoadenosine,[13] followed

by removal of isopropylidene group by 98% acetic acid. Compound Id was synthesized by reaction of trityl chloride with 8-bromoadenosine using conditions established for the tritylation of adenosine.[14]

The 5'-blocked nucleosides, thus obtained, were allowed to react with sodium hydride in dry dimethylformamide and then with TPS-Cl. The reaction products were examined by TLC. Material corresponding to each spot were isolated and its physico-chemical properties and elemental analysis determined. The results are shown in Table 1. The structure of 2'-TPS and 3'-TPS compound was confirmed further by transformation to the authentic 8,2'-S-cyclo- and 8,3'-S-cyclonucleoside.[3]

2'-TPS-5'-trityl-8-bromoadenosine (IId) (R_f 0·68) was obtained by recrystallization of the reaction product or by column chromatography on alumina. Compound IId showed IR absorption bands at 1178 and 1353 cm^{-1} corresponding to sulfonic ester and UV absorption maxima in ethanol at 265·5 nm similar to that of 8-bromoadenosine.[12] Cyclization of IId with sodium hydrogen sulfide in dimethylformamide–water media afforded 5'-trityl-8,2'-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine (5'-trityl-8,2'-S-cycloadenosine) (IVd), which could be detritylated in 80% acetic acid to give 8,2'-S-cycloadenosine (IVa). Thus compound IId was the 2'-TPS derivative. Elemental analyses of compound IId supported this conclusion.

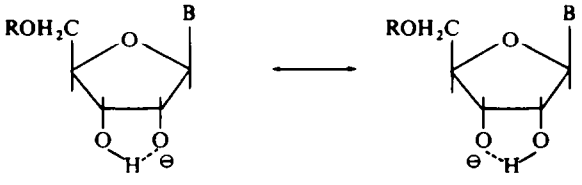
A series of reactions with compound IIId (R_f 0·40) similar to those performed for compound IId gave 8,3'-anhydro-8-mercapto-9-β-D-xylofuranosyladenine[3] (8,3'-S-cycloadenosine) (Va) via 5'-trityl-8,3'-S-cycloadenosine (Vd). Compound IIId was thus shown to be 3'-TPS-5'-trityl-8-bromoadenosine. The great difference in R_f values in silica gel TLC of two isomers, the 2'-TPS (IId) and 3'-TPS derivative (IIId), is surprising, because the 2'- and 3'-tosyl-8-bromoadenosine[3] had almost the same R_f values in thin-layer and paper chromatography. This may be due to a difference in conformation of IId and IIId caused by the interaction of the bulky trityl and triisopropylbenzene group. Examination of molecular models showed that the 2'-isomer (IId) was a more folded conformation than the 3'-counterpart.



The products from the reaction of TPS with 5′-O-acetyl-8-bromoadenosine (Ib) had R_f 0·40 and R_f 0·28 in TLC. These compounds, IIb and IIIb, were separated from each other by silica gel column chromatography and purified by recrystallization. Both compounds showed IR absorption bands at 1183 and 1180 cm^{-1} (assigned to the aryl sulfonic ester) and 1730 and 1737 cm^{-1} (assigned to the acetyl group). UV absorption maxima at 268 nm also suggested the 8-bromoadenosine chromophore, which ruled out a possible sulfonylation of the adenine ring. Deacetylation with ammonia–methanol gave 2′-TPS (IIa) and 3′-TPS-8-bromoadenosine (IIIa) and confirmed the structure of IIb and IIIb as 2′-TPS- and 3′-TPS-5′-O-acetyl-8-bromoadenosine. The elemental analysis was in agreement with this structure.

The products obtained in the sulfonylation of unprotected 8-bromoadenosine were purified by recrystallization (IIa and IIIa). Both compounds showed IR absorption bands at 1180 cm^{-1} and 1185 cm^{-1}. The UV absorption maxima at 265–268 nm resembled that of 8-bromoadenosine. Elemental analysis and the successive cyclization to 8,2′- and 8,3′-S-cyclonucleoside confirmed the structure of IIa and IIIa as 2′-TPS and 3′-TPS-8-bromoadenosine. In the sulfonylation of unprotected 8-bromoadenosine a small amount of di-TPS compound was obtained. In large scale experiments with the long reaction times often another byproduct was seen. Though the structure of this compound was not completely elucidated, its chromatographic mobility and UV absorption properties suggested that it may be a dimer containing intermolecular 8,2′- or 8,3′-ether linkages.[15]

As judged from the results in Table 1, the ratio of 2′- to 3′-isomer increased as the bulkiness of 5′-protecting group increased. In the extreme case of the 5′-trityl, 3 times as much 2′-TPS compound than 3′-isomer was formed. In the case of the 5′-benzoyl- and 5′-acetyl derivatives, the ratio 2′-TPS/3′-TPS was nearly one and the ratio was even lower with 5′-unprotected 8-bromoadenosine. The most striking fact was that even in unprotected 8-bromoadenosine 5′-TPS compound was formed only in very low yield. These results are consistent with previous reports of Martin *et al.*[16] and Gin *et al.*[17] who showed that methylation of adenosine in alkaline solution leads to far more 2′-isomer than 3′-isomer and only to a small amount of the 5′-substituted compound. These results could be explained by assuming an anion stabilized by resonance between the two hybrids, a and b in Fig 1.



If a bulky group was substituted at 5′-position of 8-bromoadenosine, 2′-OH might be more easily sulfonylated by TPS than the 3′-OH for steric reasons. This situation could be demonstrated in a molecular model showing that the 3′-OH is in strong contact with 5′-trityl group in the case of 5′-trityl-8-bromoadenosine. In contrast to this, if 5′-OH is free, the 3′-OH might be more easily substituted than 2′-OH, because of the steric interference of the bromoadenine moiety. A recent investigation of the

crystal structure of 8-bromoguanosine showed a "syn" structure for bromoguanosine,[18] which suggests the same conformation in 8-bromoadenosine. Imura *et al.*[11, 19] reported that benzylation of uridine in dimethylformamide gave only 30% of 2'-benzyl derivative and no 3'-isomer. However, in this case substitution occurred at the N^3 position before effect on sugar OH and therefore the ionized species may be different from the present case.

The fact that the 2'-TPS compound always show higher R_f values in TLC than the 3'-TPS derivatives is interesting because it suggests more packed form for 2'-TPS than for 3'-TPS compound. This point was further supported by the UV absorption properties of two isomers. Table 2 shows that difference in extinction value ε in acidic solution (ε_A) and in neutral solution (ε_N) was always positive. This is in accordance with the assumption that hypochromicity in neutral solution (due to stacking of base and TPS group) is abolished in acidic solution. Furthermore, the differences in $\varepsilon_A - \varepsilon_N$ value for 2'- and 3'-isomer (see the last column in Table 2) is 100, 400 and 700 for 5'-trityl, 5'-acetyl and unprotected nucleoside, respectively. This may suggest that the interaction of the TPS residue with the bromoadenine ring increased in the order Tr < Ac < H in 5'-position. Investigation of NMR spectra also supported this view. Chemical shifts of H^2 of 2'-isomer always appeared in the higher field compared to that of the 3'-isomer. As studied by Ts'o *et al.*,[20] the signal of H^2 of the adenine ring shows a shift to higher field when an adjacent adenine ring is in stacked position. The existence of this sort of interaction in adenosine derivative has been reported previously.[21]

Sulfonylation of 8-bromoadenosine derivatives was thus achieved selectively by using sodium hydride in dimethylformamide followed by the addition of TPS-Cl as sulfonylating agent. By this procedure the 2'- as well as 3'-TPS derivative of 8-bromoadenosine were synthesized in high yield. From the TPS compounds, the 2'-S- and 3'-S-cyclonucleosides were obtained in good overall yield. Moreover, the hitherto unknown[2, 22] 8,3'-O-cyclonucleoside, 8,3'-anhydro-8-oxy-9-β-D-xylofuranosyladenine was first synthesized. The details of this experiment will be reported in a forthcoming paper.

TABLE 1. PERCENT YIELD AND R_f VALUES IN TLC OF 5'-SUBSTITUTED 8-BROMOADENOSINE

Starting material	2'-TPS compound %	2'-TPS compound R_f[a]	3'-TPS compound %	3'-TPS compound R_f	Other products %
8-Br-5'-Tr-adenosine	70·5	0·68	24·8	0·40	4·7
8-Br-5'-Bz-adenosine	46·8	0·60	35·1	0·45	18·1
8-Br-5'-Ac-adenosine	43·1	0·40	42·7	0·28	14·2
8-Br-adenosine	38·0	0·21	44·0	0·16	18·0

[a] Solvent of TLC was in text.

EXPERIMENTAL*

Paper chromatography. Ascending chromatographies were performed on Toyo Roshi No. 51A paper in the following solvent: solvent A, water adjusted to pH 10 with conc ammonia; solvent B, n-BuOH-water, 86:14; solvent C, i-PrOH-ammonia-water, 7:1:2.

* Ultraviolet spectra were measured with a Hitachi EPS-3T recording spectrophotometer, infrared spectra with a Hitachi EPI-L spectrophotometer, and NMR spectra with a Hitachi H-6013 high resolution spectrometer operated at 60 mc with tetramethylsilane as internal standard.

TABLE 2. PROPERTIES OF UV EXTINCTION OF 2′- AND 3′-TPS-5′-SUBSTITUTED 8-BROMOADENOSINE

Compound	$\varepsilon_A-\varepsilon_N$[a]	2′-TPS$_{\varepsilon_A-\varepsilon_N}$-3′-TPS$_{\varepsilon_A-\varepsilon_N}$[b]
2′-TPS-5′-Tr-8-Br-adenosine	1200	100
3′-TPS-5′-Tr-8-Br-adenosine	1100	100
2′-TPS-5′-Ac-8-Br-adenosine	1700	400
3′-TPS-5′-Ac-8-Br-adenosine	1300	
2′-TPS-8-Br-adenosine	1500	700
3′-TPS-8-Br-adenosine	800	

[a] $\varepsilon_A-\varepsilon_N$ stands for difference of ε in acidic and neutral media.
[b] Difference of $\varepsilon_A-\varepsilon_N$ value of 2′- and 3′-TPS compounds.

TABLE 3. ELEMENTAL ANALYSIS OF TPS COMPOUNDS

Compound	Theoretical (%)			Found (%)		
	C	H	N	C	H	N
8-Br-5′-Tr-2′-TPS-adenosine	61·81	5·66	8·19	61·95	5·73	8·13
8-Br-5′-Ac-2′-TPS-adenosine	49·54	5·57	10·69	49·50	5·70	10·69
8-Br-5′-Ac-3′-TPS-adenosine	49·54	5·57	10·69	49·64	5·49	10·73
8-Br-2′-TPS-adenosine	49·01	5·59	11·42	49·27	5·63	11·17
8-Br-3′-TPS-adenosine	49·01	5·59	11·42	48·99	5·83	11·21

TABLE 4. PHYSICAL PROPERTIES OF TPS COMPOUNDS

Compound	M.p.	UV max (nm) (ε)	IR (cm^{-1})	NMR (δ)
2′-TPS-5′-Tr-8-Br-adenosine	123–124°	pH1 264 (14,900), 233 (18,600, sh)[a], 285 (6000, sh) pH7 265·5 (13,700), 233 (18,600, sh), 285 (6000, sh) pH13 265·5 (12,800), 233 (17,500, sh), 285 (5800, sh)	1178 (sulfonate)	7·96 (2-H)
3′-TPS-5′-Tr-8-Br-adenosine		pH1 264 (16,900), 233 (19,100, sh), 285 (7000, sh) pH7 264·5 (15,800), 232 (21,000, sh), 285 (7000, sh) pH13 266 (13,700), 232 (17,800, sh), 285 (7100, sh)	1180 (sulfonate)	
2′-TPS-5′-Ac-8-Br-adenosine	90–91°	pH1 264·5 (14,400), 235·5 (12,900), 286 (5900, sh) pH7 266 (12,700), 235 (11,000), 286 (5400, sh) pH13 265 (12,600), 2385 (10,900), 286 (5400, sh)	1183 (sulfonate) 1730 (carbonyl)	7·92 (2-H)
3′-TPS-5′-Ac-8-Br-adenosine	104–106°	pH1 264 (16,600), 235 (14,000), 285 (7100, sh) pH7 266 (15,300), 234·5 (13,700), 285 (7100, sh) pH13 266 (15,900), 235 (14,000), 285 (7500, sh)	1180 (sulfonate) 1737 (carbonyl)	7·98 (2-H)
2′-TPS-8-Br-adenosine	217–218°	pH1 265 (17,400), 235 (13,900), 285 (7600, sh) pH7 266 (15,900), 234·5 (13,200), 285 (7400, sh) pH13 267·5 (15,300), 238 (13,500), 285 (7200, sh)	1185 (sulfonate)	7·91 (2-H)
3′-TPS-8-Br-adenosine	185–186°	pH1 265 (16,100), 234 (12,700), 285 (6900, sh) pH7 266·5 (15,300), 233·5 (12,400), 285 (6900, sh) pH13 268 (13,500), 234 (13,400), 285 (8000, sh)	1180 (sulfonate)	8·07 (2-H)

Thin-layer chromatography. Performed on Merck Kieselgel HF 254 in solvent of $CHCl_3$-EtOH, 19:1, unless otherwise noted.

Column chromatography. Mallinckrodt silicic acid, 100 mesh, and Merck aluminium oxide, active neutral were used.

8-Bromoadenosine. Adenosine (21 g, 0·08 mole) was dissolved in a NaOAc buffer (0·5M, pH 4, 400 ml) with stirring and slight heating. Then the soln was cooled to room temp and Br_2-water (600 ml) was added. After 3 hr at room temp, the colour of the soln was discharged by addition of 5N $NaHSO_3$ and pH of the soln adjusted 7 with 5N NaOH. After cooling for 10 hr the crystalline ppt was filtered off, washed with water (50 ml), then with acetone (50 ml) and dried (yield 18·67 g). The mother liquor was evaporated to half its volume and a second crop of bromoadenosine (1·93 g) was obtained, total yield was 74·5%. A sample was further recrystallized from water and had a m.p. >200°, dec. (Found: C, 34·90; H, 3·67; N, 20·23. Calc for $C_{10}H_{12}O_4N_5Br$: C, 34·70; H, 3·49; N, 20·33%); UV: pH1, 263 nm (ε 16,600); pH7, 212 nm (ε 20,800), 265 nm (ε 15,500); pH 12, 213 nm (ε 20,000), 265·5 nm (ε 15,600). Paper chromatography: R_f (A)* 0·43, R_f (B) 0·43, R_f (C) 0·75. This sample was identical with an authentic sample.[12]

8-Bromo-5'-O-trityladenosine. 8-Bromoadenosine (7·0 g, dried over P_2O_5 in 2 mm/Hg for 5 hr at 60°) was dissolved in dry pyridine (130 ml) with stirring and heating at 60°. Trityl chloride (2·0 g) was added to this soln at this temp. After 1 hr at 60° another 2 g trityl chloride and after 2 hr, 2·13 g trityl chloride were added. The reaction was continued at 60° for 3 more hr. After cooling the mixture to room temp a yellow ppt formed which was removed by filtration. The filtrate was poured portionwise into sat $NaHCO_3$ aq. The resulting gummy material was separated from water by decantation, dissolved in chloroform (300 ml), and the chloroform layer washed with water (2 × 200 ml). After drying over $MgSO_4$ overnight, the chloroform soln was evaporated to half its volume. To this soln benzene (100 ml) was added. Gradual evaporation of this soln gave tiny needles, which were filtered off and washed with a small amount of benzene. After drying over P_2O_5 (5 hr at 60° in 2 mm/Hg) 8-bromo-5'-trityladenosine was obtained in a yield of 6·0 g (51%), m.p. was 192–193·5°. (Found: C, 59·20; H, 4·47; N, 11·71. Calc for $C_{29}H_{26}O_4N_5Br$: C, 59·19; H, 4·46; N, 11·90%); UV: pH1, 264·5 nm (ε 12,000); pH7, 264 nm (ε 10,900); pH12, 264·5 nm (ε 10,800); IR (KBr): 700 cm^{-1} (trityl); paper chromatography: R_f (B) 0·93, R_f (C) 0·96.

8-Bromo-5'-O-benzoyladenosine. To an anhyd soln (60 ml) of 8-bromo-2',3'-O-isopropylideneadenosine (5·80 g, 15 mmoles) in dry pyridine, was added benzoic anhydride (4·1 g, 18 mmoles) and the mixture kept at 2° for 2 days. To complete the benzoylation another addition of benzoic anhydride (2·17 g, 12 mmoles) was made. Examination of the reaction mixture by TLC showed incomplete reaction. Again benzoic anhydride (2·26 g, 10 mmoles) was added and the reaction mixture kept at room temp for 3 days. The soln was poured under stirring into water saturated with chloroform (200 ml) and the chloroform layer was washed with water saturated with $NaHCO_3$ (300 ml) and with water (200 ml). After drying over $MgSO_4$, the chloroform soln was evaporated to give a brownish glass, which was dissolved in benzene. Column chromatography on alumina gave the monobenzoyl derivative, the dibenzoyl derivative and starting material in this order. The yield of the monobenzoyl compound was 3·18 g. A part of this compound (1·06 g, 2·16 mmoles) was dissolved in 98% formic acid (50 ml) and the mixture was kept at room temp for one day and at 2° for 3 days. Formic acid was removed by vacuum distillation until the acid was totally removed by azeotropic distillation with anhyd EtOH. The residual yellow glass was dissolved in chloroform (3 ml) and applied to a column (2 × 10 cm) of alumina, which was developed by chloroform. 8-Bromo-5'-benzoyladenosine was obtained as a colourless glass, which was recrystallized from EtOH to give tiny prisms (204 mg), m.p. was 178–179°. (Found: C, 45·99; H, 3·78; N, 14·98. Calc for $C_{17}H_{16}O_5N_5Br \cdot 1/2C_2H_5OH$: C, 45·67; H, 4·06; N, 14·80%); UV: pH1, 264 nm (ε 15,300), 232 nm (ε 14,800); pH7, 265 nm (ε 13,900), 231 nm (ε 15,300); pH13, 266 nm (ε 14,900), 232 nm (ε 16,500); IR(KBr): 1715 cm^{-1} (carbonyl); paper chromatography: R_f (A) 0·41, R_f (B) 0·85. R_f (C) 0·96.

8-Bromo-5'-O-trityl-2'- and 3'-triisopropylbenzenesulfonyladenosine. 5'-O-Trityl-8-bromoadenosine (1·764 g, 3 mmoles) was dissolved in DMF (30 ml) and cooled to −15°. To this soln was added NaH (180 mg, 3·6 mmoles, containing 50% mineral oil), washed with dry benzene and suspended in DMF (5 ml). After stirring for 15 min at −15°, TPS-Cl (1·09 g, 3·6 mmoles) was added and the stirring continued for 2 hr at −15°. The mixture was poured into water (500 ml) saturated $NaHCO_3$ aq with stirring. The ppt was filtered off, washed with ice water, and dried over P_2O_5 in a desiccator. The white powder (2·06 g), thus obtained, was dissolved in chloroform (10 ml) and applied to a column (3 × 20 cm) of alumina. The column was eluted with chloroform and the fractions were examined by TLC. The fractions were pooled accordingly

* R_f(A) stands for R_f value obtained in the solvent A.

and evaporated *in vacuo*. The 2'-TPS compound was obtained as a pink glass (1·23 g). Recrystallization of this material from benzene gave prisms (785 mg).

The 3'-TPS compound was obtained as colourless glass (yield 210 mg), which was recrystallized from n-hexane to give a white powder (210 mg). From the late-eluting fractions a mixture of 2'- and 3'-TPS compound was obtained, yield 620 mg. The properties of the 2'- and 3'-compound were shown in Tables 3 and 4.

8-*Bromo*-5'-O-*benzoyl*-2'- *and* 3'-*triisopropylbenzenesulfonyladenosine*. To an anhyd soln of 8-bromo-5'-benzoyladenosine (90·4 mg, 0·2 mmole) in DMF (5 ml) was added NaH (12 mg, 0·24 mmoles, containing 50% mineral oil, washed with benzene, at −15°. After stirring for 15 min at −15°, TPS-Cl (72·4 mg, 0·24 mmole) was added and stirring continued for one hr at −15°. The reaction mixture was poured into water (200 ml) and the ppt was extracted with chloroform (100 + 50 ml). The organic phase was washed with water (100 ml) and dried over $MgSO_4$. Then the solvent was evaporated to give a mixture of 2'- and 3'-TPS compound as a colourless glass (106 mg). Properties were summarized in Table 3.

8-*Bromo*-5'-O-*acetyl*-2'- *and* 3'-*triisopropylbenzenesulfonyladenosine*. To an anhyd soln of 8-bromo-5'-acetyladenosine (1·164 g, 3·0 mmoles) in DMF (20 ml; cooled to −15°) was added NaH (180 mg, 3·6 mmoles, containing 50% mineral oil), washed well with benzene and suspended in DMF (5 ml). After 15 min stirring at −15°, TPS-Cl (1·087 g, 3·6 mmoles) was added into this soln and stirring continued for 3 hr at −15°. The reaction mixture was poured into water (100 ml) under stirring. The ppt was extracted with chloroform (50 ml × 3). The chloroform phase was washed with water (100 ml × 2), dried over $MgSO_4$ and the solvent evaporated to yield a yellow glass (1·81 g). It was dissolved in chloroform (10 ml) and applied to a column (2·5 × 39 cm) of silica gel. The column was eluted with chloroform, and chloroform containing 0·5% EtOH. The eluate was examined by TLC and fractions having R_f 0·40 and 0·28 were pooled. On evaporation the fractions gave the 2'-TPS (595 mg) and the 3'-TPS (390 mg) compound. The 2'-isomer was recrystallized from benzene (488 mg). The 3'-isomer precipitated from benzene by addition of n-hexane to give 362 mg white powder. From the middle fractions a mixture of both isomers was obtained (550 mg). The properties and analytical data of compounds thus obtained was summarized in Tables 3 and 4.

Deacylation of these substances with methanolic ammonia gave 8-bromo-2'-TPS- and 3'-TPSadenosine, described below, respectively.

8-*Bromo*-2'- *and* 3'-*trilsopropylbenzenesulfonyladenosine*. (i) To an anhyd soln of bromoadenosine (1·04 g, 3·0 mmoles) in dry DMF (20 ml) at −15° was added NaH (180 mg, containing 50% mineral oil), washed with dry benzene and suspended in DMF (5 ml). After 15 min TPS-Cl (1·09 g, 3·6 mmoles) was added and stirring was maintained for 3 hr. The reaction mixture was poured into water (400 ml) with stirring. After 12 hr the ppt was collected, washed with water and dried in a desiccator. A white solid (1·763 g) was obtained, which was dissolved in chloroform (50 ml) and applied to a column (3 × 20 cm) of silica gel. The column was eluted with chloroform, chloroform containing 0·5% EtOH, and finally with chloroform containing 1% EtOH. Fractions with material having R_f 0·21 and R_f 0·16 on TLC were pooled and evaporated. The early fractions gave 392 mg of a glass, which was recrystallized from benzene to give 2'-TPS compound (371 mg). The latter fractions were evaporated (467 mg) and recrystallized from benzene to give 3'-TPS compound (318 mg). From the middle fractions 263 mg of mixture containing 2'- and 3'-TPS compounds was isolated. The properties and analytical data were summarized in Tables 3 and 4. (ii) To an anhyd soln of 8-bromoadenosine (6·92 g, 2 mmoles) in DMF (180 ml) was added NaH (1·20 g, 24 mmoles, containing 50% mineral oil), washed with benzene and suspended in DMF (10 ml). After stirring of the soln at −15° for 15 min, TPS-Cl (6·64 g, 22 mmoles) was added. Reaction mixture was stirred for 2 hr at −15° and poured into saturated $NaHCO_3$ aq (800 ml). After 30 min stirring the ppt was filtered off and dried in a desiccator under 50°. TPS compounds were obtained in a yield of 11·95 g as white powder. Recrystallization from benzene (150 ml) gave 3·17 g of 2'-TPS compound, which was identical with a sample obtained above. From the mother liquor a glass was obtained by evaporation of solvent. Recrystallization from MeOH (100 ml) gave 2·84 g of 3'-TPS compound. The mother liquor was evaporated and the residue crystallized from benzene to give 1·15 g of 2'-TPS as second crop. The final residue, which was obtained from this mother liquor by evaporation, was crystallized from MeOH to give 1·24 g of 3'-TPS compound. The properties of these compounds are listed in Tables 3 and 4.

5-*Trityl*-8,2'-*anhydro*-8-*mercapto*-9-β-*D*-*arabinofuranosyladenine*. N_2 gas was bubbled through DMF (130 ml) for 30 min under exclusion of moisture. In it was dissolved 2'-TPS-5'-trityl-8-bromoadenosine (1·27 g) and N_2 was further bubbled through for 10 min under cooling. To this soln was added 40% NaHS aq. The reaction mixture was kept at 70–75° for 22 hr. At the end of the reaction the colour of the mixture turned blue-green. The mixture was poured into ice water (150 ml) and stirred for 3 hr. The ppt was filtered

off and dried over P_2O_5 in vacuum. Recrystallization of the white powder from acetone (30 ml) gave needles, m.p. 221–222°, yield 650 mg, 83%. (Found: C, 65·89; H, 4·92; N, 13·16. Calc for $C_{29}H_{25}O_3N_5S·1/2H_2O$: C, 65·40; H, 4·93; N, 13·15%); UV: pH1, 279 nm (ε 17,300), 287 nm (ε 16,100, shoulder); pH7, 271 nm (ε 16,500, shoulder), 277 nm (ε 17,700); pH13, 271 nm (ε 15,600, shoulder), 277·5 nm (ε 16,900); paper chromatography: R_f (B) 0·89.

8,2′-*Anhydro*-8-*mercapto*-9-β-*D*-*ribofuranosyladenine*. To an anhyd soln of 8-bromo-2′-triisopropylbenzenesulfonyladenosine (609 mg, 1 mmole) in DMF (10 ml) was added freshly prepared 40% NaHS (0·4 ml, 3 mmoles). The reaction mixture was kept at 60–70° for 14 hr. The yellow reaction mixture was then neutralized with NHCl and evaporated to dryness under reduced press. The residual solid was taken up in water (10 ml) and insolubles were removed by centrifugation. The supernatant was evaporated *in vacuo*, water (15 ml) was added, and stored in a refrigerator overnight. Colourless crystals of 8,2′-S-cycloadenosine were collected by filtration, washed with acetone (5 ml) and dried over P_2O_5; m.p. 210–213°, melted once at 140–150° and solidified at 173°; yield 59%. An analytical sample was further recrystallized from water. (Found: C, 40·78; H, 4·49; N, 23·45. Calc for $C_{10}H_{11}O_3N_5S·2/3H_2O$: C, 40·95; H, 4·24; N, 23·88%); UV: pH1, 277 nm (ε 20,100); pH7, 275·5 nm (ε 20,300), 220·5 nm (ε 19,300); pH13, 276 nm (ε 20,300); 220·5 nm (ε 19,300); paper chromatography: R_f (A) 0·41, R_f (B) 0·31, R_f (C) 0·56; NMR: 8·09 (s, H^2), 7·08 (s, NH_2), 6·51 (d, H^1, $J_{1'-2'}$ 6·6 cs). These properties were identical with those reported in Ref 3 and the sample was confirmed to be same as an authentic specimen.

8,3′-*Anhydro*-8-*mercapto*-9-β-*D*-*xylofuranosyladenine*. To an anhyd soln of 8-bromo-3′-triisopropylbenzenesulfonyladenosine (609 mg, 1·0 mmole) in DMF (10 ml) was added freshly prepared 40% NaHSaq (0·4 ml, 3·0 mmole). The mixture was kept at 60–70° for 14 hr. The brownish reaction mixture was neutralized with NHCl and evaporated to dryness *in vacuo*. The residue was dissolved in water and insoluble material was removed by centrifugation. The supernatant was evaporated *in vacuo* and the residue taken up in water. Crystals formed on standing at 2°. The colourless needles were collected by filtration, washed with water (5 ml) and acetone (5 ml). The crystals melted at 166–175°, solidified at 180–185°, and melted again at 272–290° with decomposition, yield was 120 mg, 42·5%. (Found: C, 40·32; H, 4·46; N, 22·89. Calc for $C_{10}H_{11}O_3N_5S·H_2O$: C, 40·12; H, 4·38; N, 23·40%); UV: pH1, 283 nm (ε 22,000), 292 nm (ε 21,000); pH7, 225 nm (ε 17,000), 282·5 nm (ε 22,000), 290 nm (ε 15,000), 276 nm (ε 16,000, shoulder); pH13, 278 nm (shoulder), 284·5 nm (ε 22,000), 293 nm (ε 16,000, shoulder); paper chromatography: R_f (A) 0·38, R_f (B) 0·36, R_f (C) 0·55; NMR: 8·08 (s, H^2), 7·10 (s, NH_2), 5·84 (s, $H^{1'}$, $J_{1'-2'}$ 0 cs). These properties were the same as reported in Ref 3. The sample was identical with an authentic sample.[3]

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