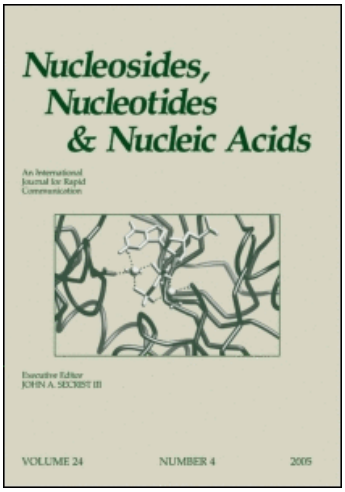


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# Studies on the Preparation, Isolation, and Reactions of Adenosine Schiff Bases

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# STUDIES ON THE PREPARATION, ISOLATION, AND REACTIONS OF ADENOSINE SCHIFF BASES

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Abstract. A systematic study of the preparation, isolation, and reactions of adenosine Schiff bases is presented. Schiff bases of nucleosides can be prepared and isolated, but the reaction appears to be specific for 2',3'-O-isopropylidene adenosines. The use of nucleoside Schiff bases as synthetic intermediates is not yet a viable process.



## INTRODUCTION

The synthesis of adenosine (1) derivatives alkylated at N-6 has gained importance in recent years with the discovery that certain derivatives have potent biological properties. $N^6$-Cyclohexyladenosine (CHA, 2), for example, has nanomolar affinity at adenosine "inhibitory" ($A_1$, $R_i$) receptors.[1,2] Synthesis of compounds such as CHA commonly involves displacement of a suitable leaving group "L" with the desired amine;[3] another popular route involves alkylation at N-1 followed by rearrangement (Scheme 1).[4] Both methods work well for most applications, however, educts are often expensive in the former route and removal of amine salts can be tedious; direct alkylation of the nucleoside is not always successful and can lead to mixtures in the latter route.[5]

In principle, reductive alkylation would be a desirable alternative, but the only reported direct reductive alkylation of adenosine requires careful pH control, and copious quantities of aldehyde and reducing agent are necessary owing to attendant aldehyde reduction.[5] Preparation and



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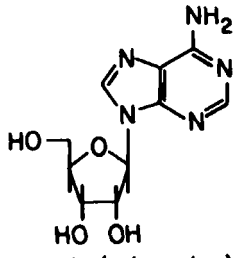
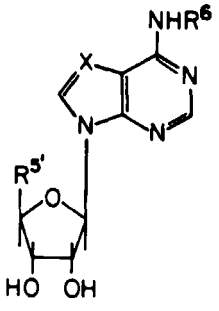
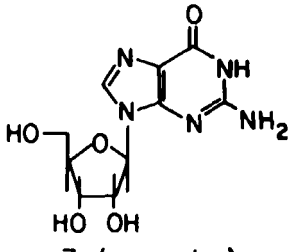


1 (adenosine)

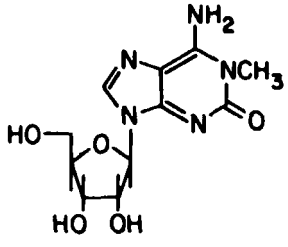


2

| | $R^6$ | X | $R^{5'}$ |
|---|---|---|---|
| CHA | $c\text{-}C_6H_{11}$ | N | $CH_2OH$ |
| tubercidin | H | CH | $CH_2OH$ |
| NECA | H | N | CONHEt |
| R-PIA | (R)-$PhCH_2CH(CH_3)$ | N | $CH_2OH$ |
| adenosine 5'-carboxylic acid | H | N | $CO_2H$ |
| 5'-chloro-5'-deoxyadenosine | H | N | $CH_2Cl$ |



3 (guanosine)

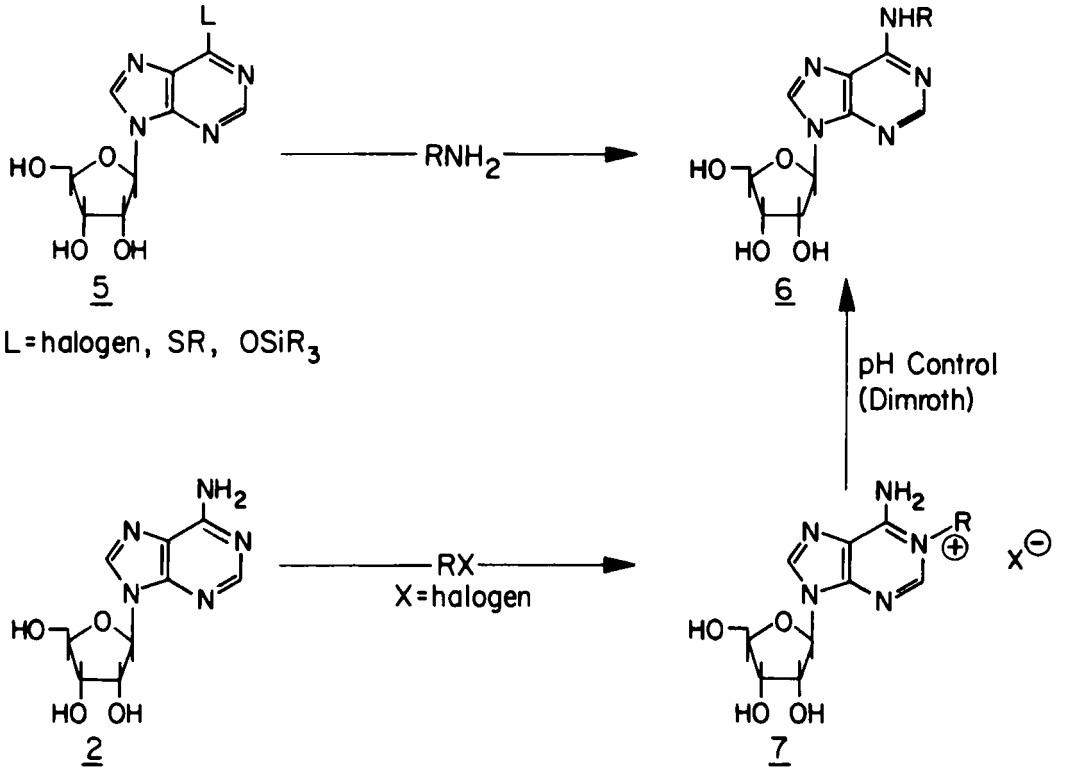


4 (doridosine)

CHART

isolation of the intermediate Schiff base as a first step followed by reduction in a second step might avoid these problems, but only one nucleoside Schiff base has been reported in the synthetic literature.[6,7]

The present report describes our studies on the preparation, isolation, and reactions of nucleoside Schiff bases. This is the first systematic study of Schiff bases derived from nucleosides.

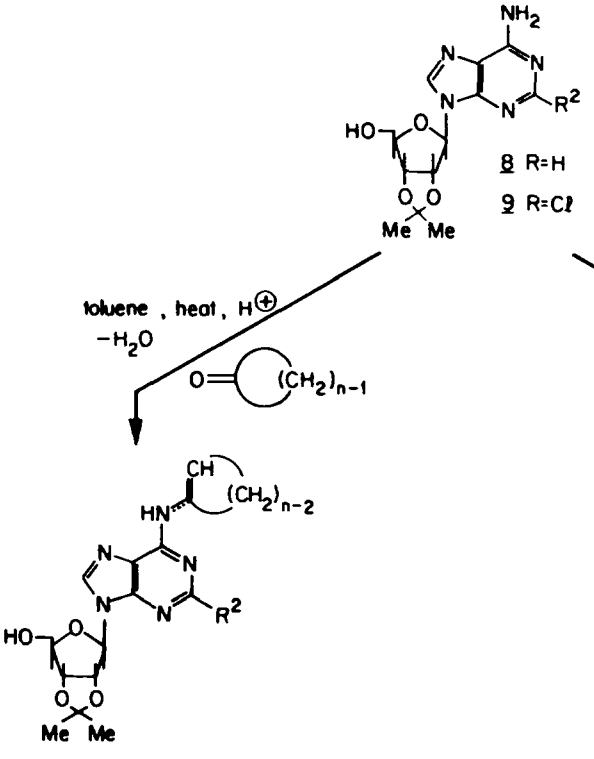



SCHEME 1

## RESULTS AND DISCUSSION

### Preparation of Schiff Bases

Work began with the 2',3'-O-isopropylidene derivative of adenosine (8), protected to guard against the possibility of reaction of the carbonyl compound with the 2',3'-hydroxyls. A variety of aldehydes reacted readily with 8 in refluxing toluene (with the removal of water and with an acid catalyst) to provide the desired Schiff bases (Scheme 2), with no evidence of trans-ketalization. The Schiff bases were somewhat stable to isolation and purification via rapid normal phase silica gel chromatography, but they were readily hydrolyzed in water or upon adsorption onto silica gel. The NMR, TLC, and HPLC data for the crudes led us to expect high yields of the Schiff bases - the low yields apparently reflect hydrolysis during chromatography.[8] Mixtures of $N^6$-isomers were apparently obtained, based upon NMR evidence.[9]




| | $R^2$ | n | Yield[a] |
|---|---|---|---|
| 18 | H | 5 | b |
| 19 | H | 6 | 21% |
| 20 | H | 8 | <10%[c] |
| 21 | H | 9 | b |
| 22 | H | 10 | 6%[c,d] |

[a] Yield refers to isolated, characterized Schiff bases.
[b] No Schiff base formation observed.
[c] Reaction is very slow.
[d] Yield refers to reaction in xylene; no reaction observed in toluene.

| | $R^2$ | $R^6$ | Yield[a] |
|---|---|---|---|
| 10 | H | Ph | 18%[b] |
| 11 | H | $4NO_2Ph$ | 13% |
| 12 | H | 4MeOPh | c |
| 13 | H | $4CF_3Ph$ | 51% |
| 14 | H | $n\text{-}C_5H_{11}$ | 29% |
| 15 | H | $c\text{-}C_6H_{11}$ | 56% |
| 16 | H | $Ph_2CH$ | c |
| 17 | Cl | $4CF_3Ph$ | 50% |

[a] Yields refer to isolated, characterized Schiff bases.
[b] Isolable, but easily hydrolyzed.
[c] Schiff base forms, but hydrolyzes completely upon chromatographic workup.

SCHEME 2

The reaction with cyclic ketones was also examined, but only cyclohexanone afforded a significant yield of "Schiff base" (Scheme 2). In this case, the double bond was predominantly endocyclic in the isolated product; mixtures of double bond isomers were obtained in other cases.[9] The reaction with cyclohexanone was more sluggish than with aldehydes, but the product was more stable. Finally, ketals were less reactive than the corresponding ketones.

Results with modified sugar and base portions were disappointing. Little or no Schiff base formation was observed under a variety of conditions wherein both educt and product were stable. Educts included adenosine, adenine, 2',3',5'-tri-O-silylated (TMS or TBDMS) adenosine, tubercidin, and the 2',3'-O-isopropylidene derivatives of guanosine, doridosine, 5'-chloro-5'-deoxyadenosine, tubercidin, NECA, R-PIA, adenosine 5'-carboxylic acid, and 5'-O-acetyl adenosine. In situ generation of $N^6$-TMS derivatives followed by reaction with aldehydes was unsuccessful in driving the process to completion. However, the 2',3'-O-isopropylidene derivative of 2-chloroadenosine (9) gave a 50% yield of Schiff base, showing some generality of this method for adenosines. Reasons for the special reactivity of 2',3'-O-isopropylidene adenosines are unknown. The limited solubility of some of these nucleosides in toluene may be a factor,[10] but the Schiff bases are not isolable in better solvents such as, for example, ethanol.

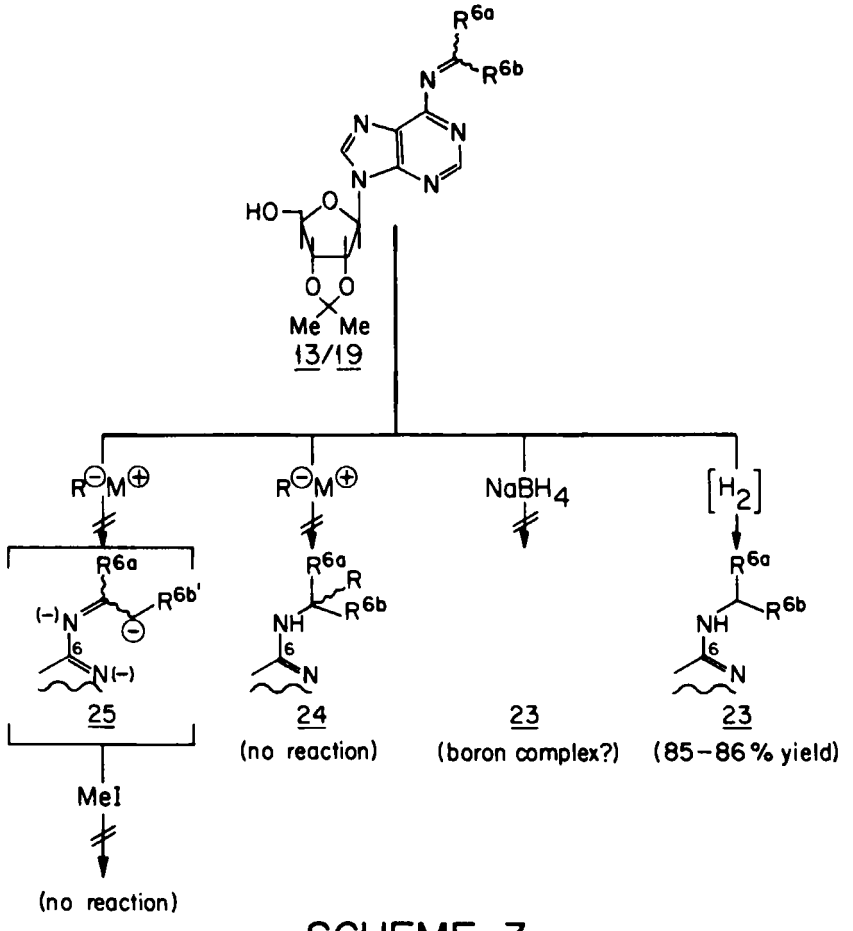


## Reactions of Schiff Bases

Reduction of aldehyde imine 13 proceeded readily under transfer hydrogenation conditions (Pd/C, 85% yield),[11] but ketone "imine" 19 required low pressure (50 psig) catalytic hydrogenation conditions (86% yield). An apparent boron complex formed with attempted $NaBH_4$ reduction, and no addition to the imine was observed with Grignard reagents or organolithium compounds. Finally, anion formation followed by alkylation was unsuccessful (see Scheme 3).

## CONCLUSIONS

Schiff bases of nucleosides can indeed be prepared and isolated. However, the use of nucleoside Schiff bases as synthetic intermediates is not yet a viable process. While reduction of the Schiff bases is




SCHEME 3

facile, overall yields for the stepwise reductive alkylation sequence are poor, apparently owing to instability of the Schiff bases.

## EXPERIMENTAL

General Methods. Toluene was dried over 4Å molecular sieves.[12] Spectra were recorded with the following instruments as indicated: infrared (IR), KBr, Nicolet FT, reported in $cm^{-1}$; ultraviolet (UV), MeOH, Cary 118, $\lambda_{max}$ reported in nm; proton nuclear magnetic resonance ($^1H$ NMR), 90 MHz, Varian EM-390 (or 200 MHz, Varian XL-200, as noted), reported as parts per million downfield from internal $Me_4Si$, with

couplings (J) in Hz (only major signals are reported); low resolution mass (LRM), electron ionization, Finnigan 4521, reported as m/z (relative intensity); high resolution (exact) mass (HRM), electron ionization, VG 7070E; optical rotation (OR), Perkin-Elmer 141. Elemental analyses were determined at Warner-Lambert/Parke-Davis.

Analytical liquid chromatography (ALC) was performed with a Waters system consisting of 2 or 3 M-45 pumps, a U6K injector, a Model 680 automated gradient controller, and either a DuPont variable wavelength UV spectrophotometer operating at 254 or 280 nm or an Altex fixed wavelength detector (254 nM), Model 153. All final products were homogeneous by ALC using the following conditions. Columns employed: (A) Altex Ultrasphere - Octyl, 5 μm, 4.6 x 250 mm; (B) Whatman Partisil PXS 10/25 ODS-2, 10 μm, 4.6 x 250 mm; (C) Hamilton PRP-1, 10 μm, 4.1 x 150 mm; (D) Alltech Silica, 10 μm, 4.6 x 250 mm. Solvents: (A) 1/1/1 $H_2O$/MeOH/MeCN; (B) 3/1 MeOH/$H_2O$; (C) 1/1 $CHCl_3$/EtOAc.

Preparative liquid chromatography (PLC) was performed on a Waters Prep 500A instrument under normal phase conditions unless otherwise noted. Analytical thin layer chromatography (TLC) was done with pre-coated glass plates (EM reagents silica gel 60 F-254).

Evaporations were performed under reduced pressure. Pressurized hydrogenations were carried out on a Parr apparatus. All reactions were monitored by TLC and/or ALC. Isolated compounds were foams or glasses unless otherwise noted.



General Procedure for Schiff Base Formation (Illustrated for 8 → 13)

A suspension of 2',3'-O-(1-methylethylidene) adenosine (5.0 g, 16.3 mmol), 4-(trifluoromethyl)benzaldehyde (6.39 g, 225 mol%), and 7,7-dimethyl-2-oxobicyclo[2,2,1]heptane-1-methanesulfonic acid (0.5 g, 12 mol%) in toluene (250 mL) is heated at reflux for 16 hours with water removal via Dean-Stark. The resulting yellow solution is evaporated in vacuo to a yellow glass which is purified by PLC (4/1 ethyl acetate/ isooctane). The major isolated fraction is evaporated in vacuo to a white foam (13): IR 2990, 2940, 1615, 1585, 1475, 1375, 1325, 1215, 1165, 1140, 1095; $^1H$ NMR ($CDCl_3$) δ 8.8-8.4 (br s,1H), 8.25 (br s,2H), 7.65 (br s,4H), 7.0-6.7 (br s,<1H), 6.2 (s,1H), 5.8-5.4 (m,1H), 5.4-5.1 (m,1H),

4.5-4.2 (m,1H), 4.0-3.3 (m,2H), 1.55 (s,3H), 1.35 (s,3H); LRM 464 (2), 374 (6), 320 (6), 292 (8), 120 (100); UV[13] $\lambda_{max}$ 263, $\epsilon$ 16600.

Anal. calcd. for $C_{21}H_{20}F_3N_5O_4$: C, 54.4; H, 4.4; N, 15.1.

Found: C, 54.4; H, 4.5; N, 15.4.

An acid catalyst is necessary. Moisture must be carefully excluded during the preparation, isolation, and reaction of these Schiff bases.

General Procedure for Schiff Base Reduction

(A) Transfer Hydrogenation: This method is successful only with aldehyde derived Schiff bases. The method has been described by Rapoport.[11]

(B) Parr Hydrogenation (Illustrated for 19 → 23): A mixture of 19 (1.00 g, 2.58 mmol), methanol (75 mL), and 5% Pt-C (0.1 g, 10 wt%) is shaken under 50 psig hydrogen pressure for 17 h which no further hydrogen uptake is observed. Filtration of the mixture through super-cel followed by evaporation of the filtrate in vacuo affords a yellow glass. Column chromatography (4/1 ethyl acetate/isooctane, normal phase $SiO_2$) affords $N^6$-cyclohexyl-2',3'-O-(1-methylethylidene)adenosine (23, 0.86 g, 86%). Proof of structure is based upon comparison with authentic material[3a,14,15] ($^1$H NMR, IR, LRM, ALC, and TLC). Physical data are reported below.

Physical Data for Schiff Bases

11: IR 2980, 2940, 1615, 1585, 1525, 1476, 1410, 1375, 1345, 1215; $^1$H NMR ($CDCl_3$) $\delta$ 9.7-9.5 (d,J = 10,1H), 8.5 (s,1H), 8.3 (s,1H), 8.1 (s,1H), 7.95 (s,1H), 7.5 (s,1H), 7.4 (s,1H), 6.6-6.5 (d,J = 10,1H), 6.4 (s,1H), 5.7-5.5 (m,2H), 5.0-4.8 (m,1H), 4.3-4.0 (m,2H), 1.7 (s,3H), 1.4 (s,3H); LRM 441 (3), 351 (19), 297 (13), 269 (15), 121 (12), 120 (100), 93 (14); UV[13] $\lambda_{max}$ 264, $\epsilon$ 8200.

Anal. calcd. for $C_{20}H_{20}N_6O_6$: C, 54.5; H, 4.6; N, 19.1.

Found: C, 54.3; H, 4.6; N, 18.9.

14: IR 2960, 2930, 2880, 1670, 1615, 1580, 1470, 1370, 1215; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.55-8.2 (m,2H), 6.2 (br s,1H), 5.4-5.2 (m,1H), 5.2-4.8 (m,3H), 4.3-4.0 (m,1H), 3.6-3.4 (m,2H), 2.0-0.6 (br m,17H); LRM (9) 332 (15), 256 (12), 242 (20), 228 (11), 218 (31), 217 (34), 216 (16), 188 (31), 174 (47), 160 (100).[16]

15: IR 2990, 2930, 2850, 1680, 1615, 1580, 1475, 1375; $^1$H NMR ($CDCl_3$) δ 8.25 (s,1H), 7.65 (s,1H), 7.1 (s,1H), 5.7-5.6 (d,J = 10,1H), 5.2-5.1 (m,3H), 4.35 (s,1H), 4.0-3.4 (br m,3), 2.2-0.9 (br m,16H); LRM 401 (20), 229 (100), 228 (38), 218 (16), 213 (11), 200 (13), 187 (13), 186 (47), 173 (12), 164 (24), 136 (36), 135 (64), 121 (18); UV[13] $\lambda_{max}$ 285, ε 12600; OR[13] $[\alpha]_D^{20}$ -70.6° (c 1.17, MeOH).

HRM calcd. for $C_{20}H_{27}N_5O_4$: 401.206; Found: 401.206.

17: mp 158.5-161.5°C; IR 2965, 1619, 1583, 1327; $^1$H NMR (200 MHz, $CDCl_3$) δ 9.6 (br s,1H), 8.37 (s,1H), 8.2-7.0 (multiple signals), 6.50 (d,1H,J = 10), 6.29 (s,1H), 5.6-5.4 (m), 4.86 (d,1H,J = 10), 4.10 (d,1H, J = 10), 3.94 (t,1H,J = 10), 1.67 (s,3H), 1.44 (s,3H); LRM 498 (14), 328 (38), 154 (100).[16]

19: IR 2930, 1670, 1610, 1585, 1470; $^1$H NMR (DMSO-$d_6$) δ 8.6 (s, 1H), 8.35 (s,1H), 8.2 (s,1H), 6.2-6.0 (br d,J = 4.5,2H), 5.4-5.2 (m,1H), 5.2-5.0 (t,J = 7.5,1H), 5.0-4.8 (m,1H), 4.3-4.0 (br m,1H), 3.6-3.4 (t, J = 7.5,2H), 2.4-1.0 (m, 14H); LRM 387 (2), 216 (13), 215 (66), 187 (27), 186 (55), 147 (11), 136 (15), 119 (12), 69 (25), 43 (100); UV[13] $\lambda_{max}$ 285, ε 14500; OR13 $[\alpha]_D^{20}$ -64.7° (c 0.68, DMF).[16]



N$^6$-Cyclohexyl-2',3'-O-(1-methylethylidene) adenosine (23):

IR 2930, 2850, 1615, 1585, 1425; $^1$H NMR (DMSO-$d_6$) δ 8.25 (s,1H), 8.1 (s,1H), 7.4-7.6 (d,J = 7.5,1H), 7.25 (br s,1H), 6.15-6.0 (d,J = 3,1H), 5.45-5.0 (m,2H), 5.0-4.8 (m,1H), 4.3-3.9 (br m,2H), 3.6-3.4 (t,J = 4.5, 2H), 2.1 (br m,17H); UV $\lambda_{max}$ 268, ε 12800.[3a,14,15]

2-Chloro-2',3'-O-(1-methylethylidene) adenosine (9):

This material was prepared in quantitative yield by the method of Hampton[14,17] from commercially available 2-chloroadenosine (Sigma Chemical Co.): IR 2991, 1651, 1596, 1348, 1314, 1218, 1105; $^1$H NMR (200 MHz, $CDCl_3$) δ 7.9 (s,1H), 7.0 (s,2H), 5.9 (d,1H,J = 4.5), 5.2 (t, 1H, J = 6), 5.1 (d,1H,J = 6), 4.5 (s,1H), 4.0 (d,1H,J = 12), 3.8 (d,1H, J = 12), 1.6 (s,3H), 1.4 (s,3H); LRM 342 (16), 252 (23), 198 (28), 169 (100), 134 (30).

## ACKNOWLEDGMENTS

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8. Alternative isolation procedures such as crystallization, and chromatography on reverse phase silica gel, deactivated silica gel, and florisil were unsuccessful in reducing losses during purification.

9. Major NMR resonances were usually accompanied by minor signals in the same region, yet samples were homogenous by other analytical methods. This implies, but does not prove, the presence of isomeric forms.

10. Solubility cannot be the only factor as 2',3',5'-tri-O-butyldimethylsilyladenosine was recovered unchanged under normal reaction conditions.

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