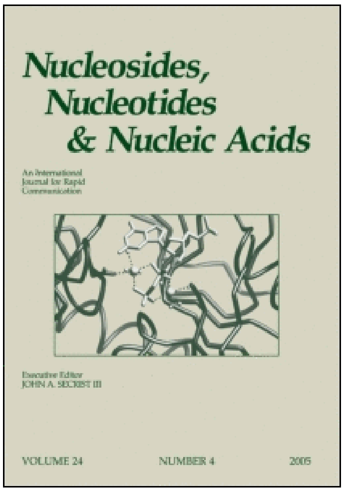

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bis(*t*BuSATE) PHOSPHOTRIESTER PRODRUGS OF 8-AZAGUANOSINE AND 6-METHYLPURINE RIBOSIDE; bis(POM) PHOSPHOTRIESTER PRODRUGS OF 2′-DEOXY-4′-THIOADENOSINE AND ITS CORRESPONDING 9α ANOMER

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▫ *As an extension of previous work with bis(POM) nucleotide prodrugs, we report the synthesis and biological evaluation in tumor cell culture of the bis(pivaloyloxymethyl) phosphotriester prodrug of slightly cytotoxic 2′-deoxy-4′-thioadenosine and its α-anomer. We have experienced need for an alternative phosphate masking group, particularly with purine nucleosides. Accordingly, we report synthesis and biological evaluation of the bis(tBuSATE) phosphotriester prodrugs of 8-azaguanosine and 6-methylpurine riboside, nucleoside analogs with moderate to significant cytotoxicity. All four prodrugs were examined in tumor cell culture in parallel with the parent nucleosides. Synthetic routes and biological data are presented.*

INTRODUCTION

In the search for new antiviral and anticancer drugs the masked nucleotide prodrug approach is now a well established strategy for getting nucleotides into cells. This is particularly important for investigating the potential of certain nucleosides that show little or no cytotoxicity because they are poorly phosphorylated in the initial activation step or because of deficiency of the appropriate nucleoside kinase. In a previous publication[1] we reported that the 5′-bis(POM)phosphate prodrugs of 8-bromo-2′-deoxyadenosine and 8-aza-2′-deoxyadenosine showed increased potencies of 10-fold and 12-fold, respectively, as inhibitors of CEM cell growth, compared to the parent nucleosides. However, in our hands the synthesis of bis(POM)phosphate prodrugs of purine nucleosides has been chronically beset by

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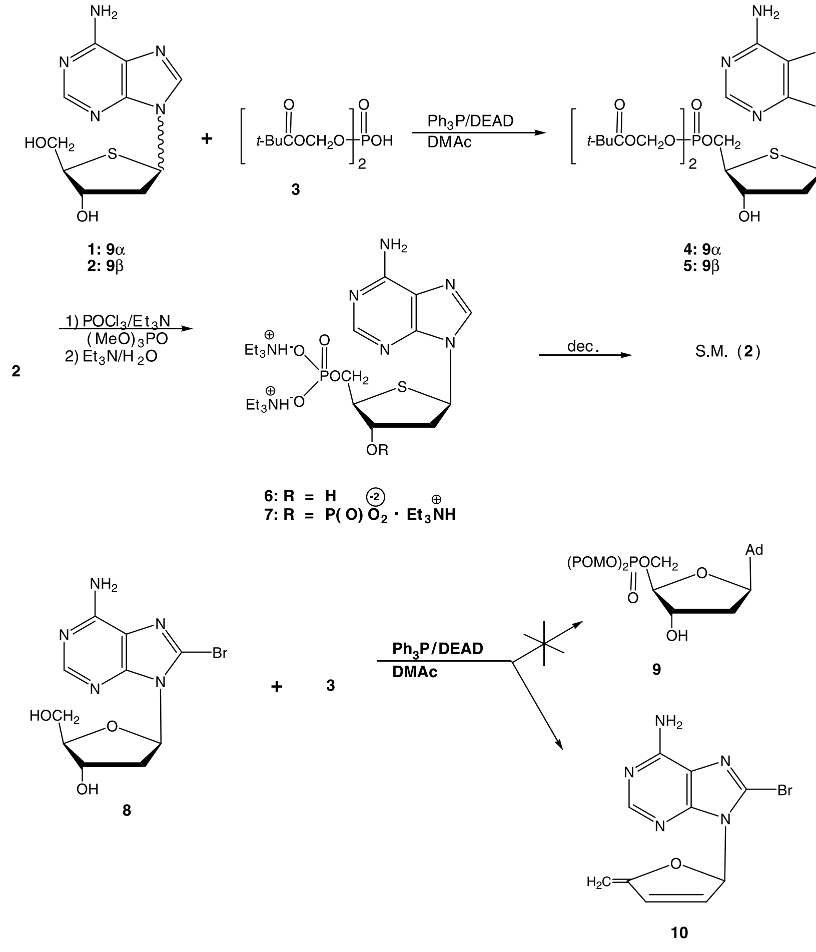
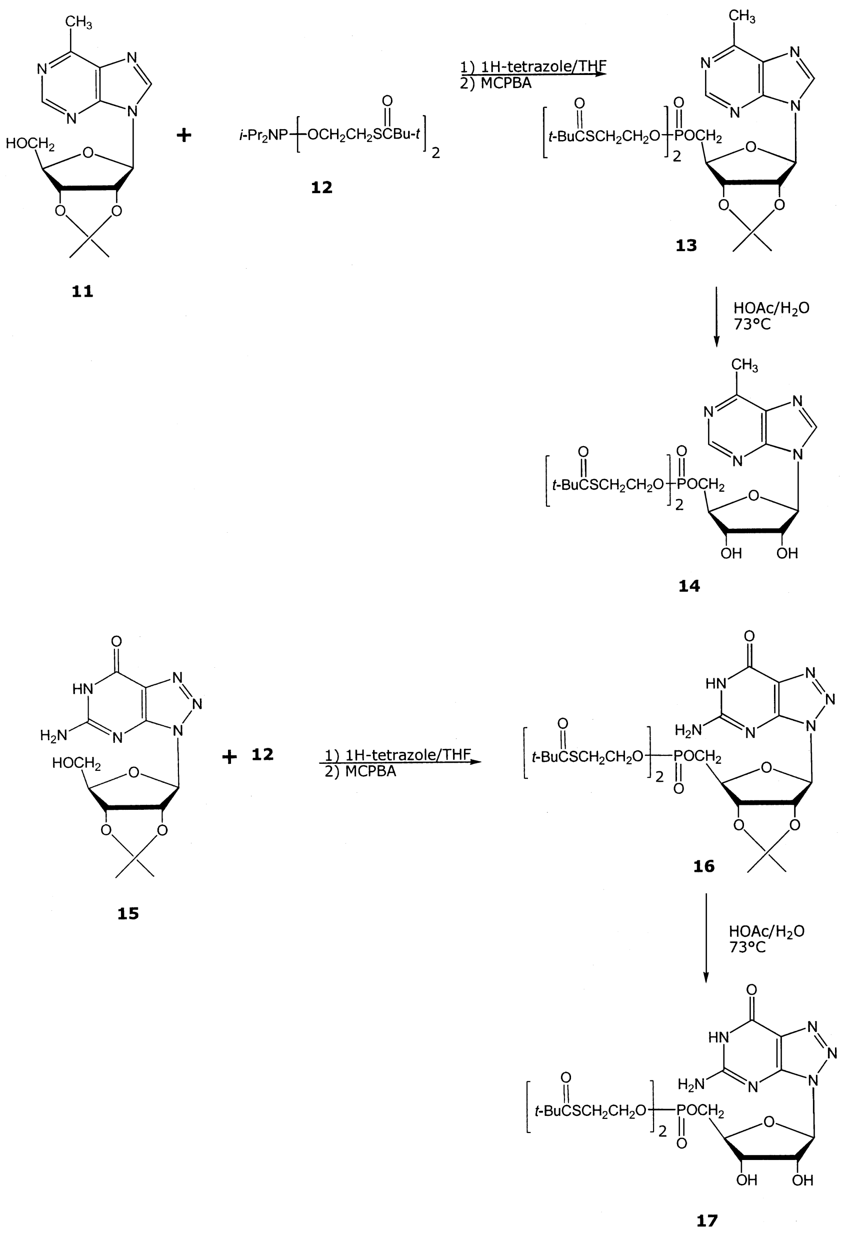
low yields for reasons that vary somewhat with the particular method used to install the bis(POM)phosphate moiety. Wishing to prepare prodrugs from nucleosides of very limited availability, we chose to pursue another biolabile masking group, specifically, S-pivaloyl-2-thioethyl-, abbreviated herein as tBuSATE.

RESULTS AND DISCUSSION

Scheme 1 outlines the conversion of 2′-deoxy-4′-thioadenosine **2** and its 9α anomer **1** to the respective bis(POM)phosphate prodrugs **5** and **4**. Nucleoside analogs **1** and **2**[2] were each reacted with bis(pivaloyloxymethyl) hydrogen phosphate 3[3] under Mitsunobu conditions by the method of Farquhar et al.,[3] which they applied to certain pyrimidine nucleosides. Silica gel column chromatography ($CHCl_3$/MeOH) easily gave prodrugs **4** and **5** as glassy foams,



SCHEME 1 bis(POM)phosphate prodrugs.



SCHEME 2 bis(*t*BuSATE)phosphate prodrugs.

albeit in yields of 28% and 15%, respectively. An alternative approach for converting **2** to **5** by direct esterification[1] of the 5′-phosphate[5] intermediate **6** was abandoned because of the instability of **6**. This result confirms the difficulty reported in the literature[4] of obtaining other 4′-thionucleotides by direct phosphorylation. This prompted us to investigate the stability of **4** and **5**. Solid samples stored at −20°C showed minimal change by TLC, but TLC solutions in

$CHCl_3$/MeOH left at room temperature showed extensive degradation on respotting. Compound 5 nearly disappeared in 6 weeks. Two degradation products from **5** tentatively identified only by TLC and MS are **2** and the corresponding methyl ether, 5′-CH_2OCH_3; MS: m/z 281 $(M + H)^+$.

Scheme 1 also shows an attempted resynthesis of our previously reported[1] bis(POM) prodrug **9** from 8-bromo-2′-deoxyadenosine **8** by the Mitsunobu coupling method so that a comparison of yields could be made with the direct esterification method using chloromethyl pivalate described previously.[1] Instead of **9**, the elimination-dehydration product **10** was isolated in a quantity sufficient for biological evaluation.

In Scheme 2, parent nucleosides 6-methyl-9-(β-D-ribofuranosyl)purine[6] and 8-azaguanosine[7] were synthesized by the referenced procedures and converted to the IP-protected compounds **11** and **15**. Bis(S-pivaloyl-2-thioethyl) *N*,*N*-diisopropylphosphoramidite **12** was obtained as a crude oil by a multistep literature procedure.[8] Reaction of excess **12** with **11** and **15**, catalyzed by 1*H*-tetrazole and followed by oxidation in situ with 3-chloroperoxybenzoic acid, gave protected nucleotides **13** and **16**, isolated by column chromatography. Deprotection with warm 80% HOAc gave the *t*BuSATE pronucleotides **14** and **17** in yields of 42% and 64%, respectively.

BIOLOGICAL EVALUATION

TABLE 1 Toxicity of 5′-Modified Nucleoside Analogs

	Cell line							
Compound	SNB-7	DLD-1	CEM	H23	ZR-75-1	LOX	PC-3	CAKI-1
IC_{50} (mM)								
4	>100	>100	42	>100	>100	>100	>100	>100
1	>100	>100	–	>100	–	–	–	>100
5	>100	>100	13	97	37	55	>100	49
2	>100	>100	75	>100	–	–	–	>100
17	>90	>90	3	13	69	6	85	>90
8-Azaguanosine	8	11	1	5	>50	1	>50	>50
14	0.6	0.4	0.07	0.1	0.3	0.2	0.3	0.09
6-MePurine riboside	0.7	0.2	0.04	0.1	0.2	0.09	0.3	0.05
10	>200	>200	>200	>200	>200	>200	>200	>200

Cells were incubated for 72 h with various concentrations of the compounds shown in the table. The effect of compound on cell growth was determined using standard procedures. The IC_{50} is the concentration of drug required to inhibit cell growth by 50%. The numbers presented are the results from one experiment. "–" not done.

- In most cases, the cytotoxicity of the prodrug was similar to that of the parent compound.
- With the 2′-deoxy-4′-thioadenosine pair, the bis(POM) prodrug appeared to be more toxic than the parent nucleoside, though only moderately toxic. This result suggests that the monophosphate is further metabolized very inefficiently.
- With the 8-azaguanosine pair, the prodrug appeared to be less cytotoxic, and in SNB-7 and DLD-1 cells, much less cytotoxic than the parent compound. Additional investigation will be required to understand this result.
- With the 6-methylpurine riboside pair, the cytotoxicities of both compounds were very similar in all cell lines tested, suggesting that the prodrug is releasing the monophosphate in cells effectively.
- The nucleoside analog 10 was inactive in all cell lines.

CONCLUSION

Neither of the bis(POM) prodrugs **4** or **5** showed significantly enhanced activity compared with the corresponding parent nucleoside. The same was true of 6-methylpurine riboside and its bis(*t*BuSATE) prodrug **14**. An unexpected result not fully understood at this point was that the bis(*t*BuSATE) prodrug **17** was consistently less cytotoxic than its 8-azaguanosine parent in all cell lines tested. The anomalous by-product **10** was inactive in all cell lines.

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