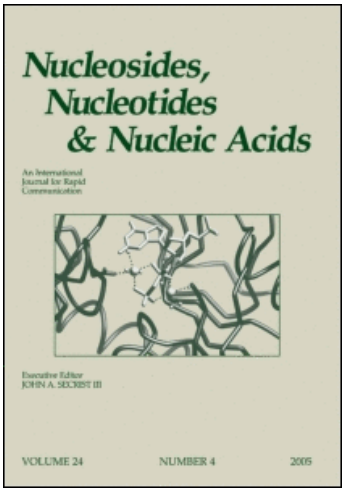

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The Regiospecific One-Pot Phosphorylation of Either the 5'- or 2'-Hydroxyl in 3'-Deoxycytidines Without Protection: Critical Role of the Base

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The Regiospecific One-Pot Phosphorylation of Either the 5′- or 2′-Hydroxyl in 3′-Deoxycytidines Without Protection: Critical Role of the Base

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ABSTRACT

We report methodology which enables direct phosphorylation of 3′-deoxycytidine exclusively either at the 5′-hydroxyl or the 2′-hydroxyl. Protection of the base is not required. Standard phosphoramidochloridates in combination with pyridine and tert-butyl magnesium chloride is employed, in which the ratio of nucleoside to Grignard reagent is crucial. These findings, which appear to be general for 3′-deoxycytidines, are not applicable to 3′-deoxyuridine or 3′-deoxyguanosine.

The use of neutral prodrugs of nucleotides (protides) to deliver nucleoside 5′-phosphates intracellularly is an intensive area of research.[1] The ability of protides to produce nucleoside-5′-triphosphates in cells when the formation of the monophosphate from the nucleoside is the rate limiting step (so-called kinase by-pass) is a particularly useful application. In the course of our research, we required the synthesis of 5′-aryloxy-phosphoramidates[2] of 3′-deoxyribonucleosides such as **1**.

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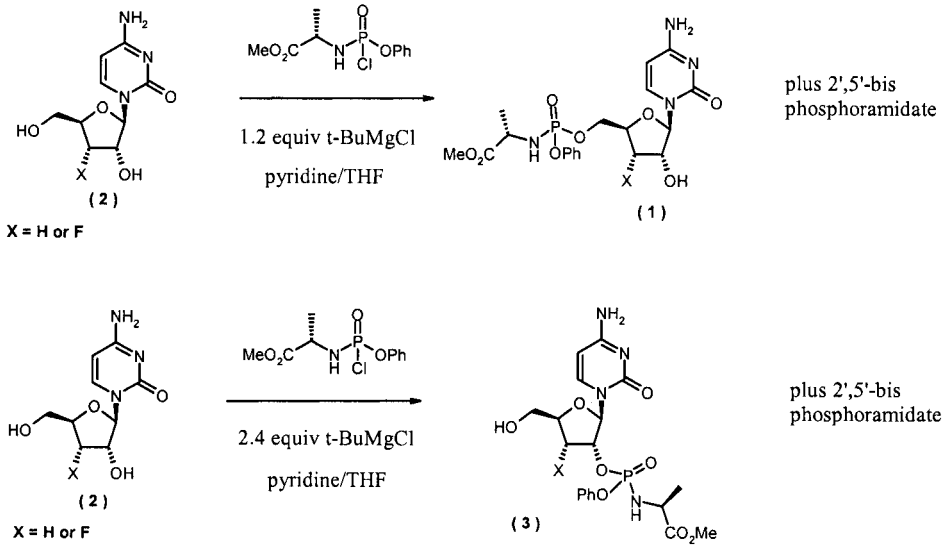
From a survey of the literature the direct phosphorylation of 3′-deoxyribonucleosides such as **2** has not been reported. We now report our findings on the phosphorylation of these systems.

Nucleosides are susceptible to both N and O phosphorylation and it is usual to protect amino functions, to ensure O-phosphorylation occurs. Noyori et al.[3] have described an alternative strategy, of selectively activating nucleosides towards O-phosphorylation by treatment of N-unprotected nucleosides with strong organometallic bases, such as t-butylmagnesium chloride. This strategy generates an equilibrium mixture of N and O deprotonated nucleosides, in which the O-deprotonated species predominates, allowing selective O-phosphorylation to take place. Although this had only been well documented for nucleosides containing a single alcohol function we anticipated that it would be applicable to diols. Under Noyori's conditions we expected nucleosides 3′-deoxy-3′-fluorocytidine (**2**, X = F) and 3′-deoxycytidine (**2**, X = H) to afford the corresponding 5′-O-phosphorylated products (**1**).

Although, as anticipated, there was no evidence for N-phosphorylation, we were surprised to observe that when **2** (X = H or F) was treated with the phosphoryl chloride in the presence of 1.2 equivalents of t-butylmagnesium chloride, selective phosphorylation on the 2′-position occurred, affording products (**3**) as pairs of diastereoisomers in a single step. These products were generally accompanied by small quantities of 2′,5′-bis phosphorylated products, which were easily removed by silica gel chromatography. We found that addition of an extra equivalent of Grignard reagent completely reversed this selectivity, exclusively giving the expected 5′ derivatives (**1**), again as a pair of diastereoisomers. Normally synthesis of structures such as (**2**) would require a circuitous protection-deprotection strategy, and our current observations represent an unprecedented reversal of the expected selectivity.



Scheme 1.

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DISCUSSION AND SUMMARY

We have found remarkable regiospecificity in the phosphorylation of 3′-deoxycytidines, solely dependent on the ratio of t-BuMgCl reagent to nucleoside. We reason that the furan oxygen and the cytosine base both contribute to stabilisation of the anion, and that the proton at the secondary hydroxyl is the most acidic,[4] and is thus preferentially removed with one equivalent of Grignard reagent. With two equivalents of base, anions of both hydroxyls are formed with the primary hydroxyl reacting preferentially on steric grounds. The nature of the base is important, as uracil and guanine bases do not show any selectivity. In these cases, the amide proton in the heterocycle is removed first and the hydroxyls then have similar pKa values.

REFERENCES

1. Naesens, L.; Snoeck, R.; Andrei, G.; Balzarini, J.; Neyts, J.; De Clercq, E. Antiviral Chem. Chemother. **1997**, *8*, 1–23.
2. a) Siddiqui, A.; McGuigan, C.; Ballatore, C.; Srinivasan, S.; De Clerck, E.; Balzraini, J. Bioorg. Med. Chem. Lett. **2000**, *10*, 381–384; b) Ballatore, C.; McGuigan, C.; De Clerck, E.; Balzraini, J. Bioorg. Med. Chem. Lett. **2001**, *11*, 1053–1056.
3. Uchiyama, M.; Aso, Y.; Noyori, R.; Hayakawa, Y. J. Org. Chem. **1993**, *58*, 373–379.
4. Velikiyan, I.; Acharya, S.; Trifonova, A.; Foldesi, A.; Chattopadhyaya, J. J. Amer. Chem. Soc. **2001**, *123*, 2893–2894, The pKa of the 2′-OH in 3′deoxyadenosine is 12.53.

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