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Use of a solid-supported coupling reagent for a selective phosphitylation of the primary alcohol of N^2-isobutyryl-2′-deoxy or 2′-*O*-methyl guanosine

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This work is dedicated to Professor Jean-Louis Imbach for his 70th birthday

Abstract—We have developed a 5′-regioselective phosphitylation of 3′,5′-OH-guanosine derivatives thanks to a solid-supported coupling reagent with either a standard or a bulky phosphine. A 5′-phosphitylation up to a 95% selectivity was obtained with a quantitative conversion of starting nucleoside. After oxidation into thionophosphotriester or phosphotriester by means of solid-supported oxidizers, the 5′-phosphorylated N^2-*i*-Bu-2′-OMe guanosines were isolated in good yields (70–80%).

1. Introduction

Nucleoside 5′-phosphates and their analogs are commonly used as biologically active compounds[1] since nucleosidic drugs are active as their 5′-triphosphate derivatives. The first phosphorylation by kinases is often the most selective and hence the most limitative step. As well explained previously,[2] the common preparation of 5′-phosphorylated nucleosides based on protection/deprotection strategy[3] is time consuming (five steps) and costly, in particular, when the nucleoside is expensive. Other methods exist for the selective 5′-phosphorylation of nucleosides,[4–9] but they present the main limitation that it is not possible to obtain a neutral compound. Thus for the synthesis of 5′-phosphorylated dimers or longer 5′-phosphorylated oligonucleotides it is required that the phosphate is protected as a phosphotriester. In order to synthesize 5′-phosphate dimers to test them as polymerase inhibitors, we developed a strategy to readily obtain the guanosine building block **I** (Fig. 1). For that purpose, N^2-isobutyryl 2′-*O*-Me guanosine was selectively 5′-phosphitylated to give the

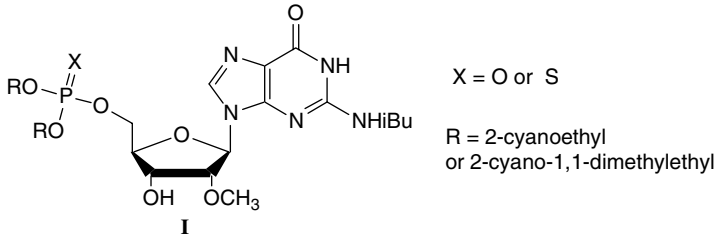

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Figure 1. Selective synthesis of 5′-phosphorylated N^2-isobutyryl-2′-*O*-methyl guanosine.

Keywords: Phosphitylation; Solid-supported reagent; Nucleotide; Phosphoramidite.
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corresponding phosphitetriester, which was finally oxidized, keeping the 3′-hydroxyl free for a next coupling.

Theoretically, the 5′-hydroxyl being a primary alcohol, the phosphitylation occurs preferentially on it. Nevertheless since the phosphitylation of 3′,5′-O-unprotected nucleosides by a phosphoramidite derivative activated with tetrazole in acetonitrile is fast, a low selectivity is usually obtained.[2] Hence some 3′-O-phosphitylation also occurs in a lower amount (<10%), which is mainly converted into the 3′,5′-diphosphite nucleoside. In addition, during the course of the reaction, the amount of 5′-O-monophosphitylated nucleoside increases in comparison with the free nucleoside and it could then react to yield the 3′,5′-*O*-diphosphite nucleoside. Furthermore, this latter side reaction is difficult to handle since an excess of phosphitylating reagent (1.3 M equiv) is usually added to counterbalance its hydrolysis due to traces of water.

We hypothesized that this side reaction could be minimized if a solid-supported reagent as coupling activator is used. Actually, the heterogeneous activation of the phosphoramidite decreases the reaction rate[10] likely leading to a better regioselectivity of the more reactive primary hydroxyl group. In addition, a maximal regioselectivity should be obtained if the heterogeneous activator is used in combination with a bulky phosphitylating reagent such as bis(2-cyano-1,1-dimethylethyl)-*N*,*N*-diisopropylphosphoramidite.[11] Indeed, it has been recently shown that a high 5′-regioselectivity of 2′-deoxyribonucleosides could be reached using the bulky di-*tert*-butyldiethylphosphoramidite.[12]

Herein, we report the selective phosphitylation of N^2-isobutyryl 2′-*O*-Me guanosine using solid-supported pyridinium tosylate as a coupling reagent and either the standard bis(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite[13] **1** or the bulky bis(2-cyano-1,1-dimethylethyl)-*N*,*N*-diisopropylphosphoramidite **2** (Scheme 1).

To validate our hypothesis, we first studied the 5′-O-phosphitylation of less expensive, N^2-isobutyryl-2′-deoxyguanosine using polyvinyl-pyridinium tosylate (PVPtos)[10] or tetrazole with phosphine **1** or **2**.

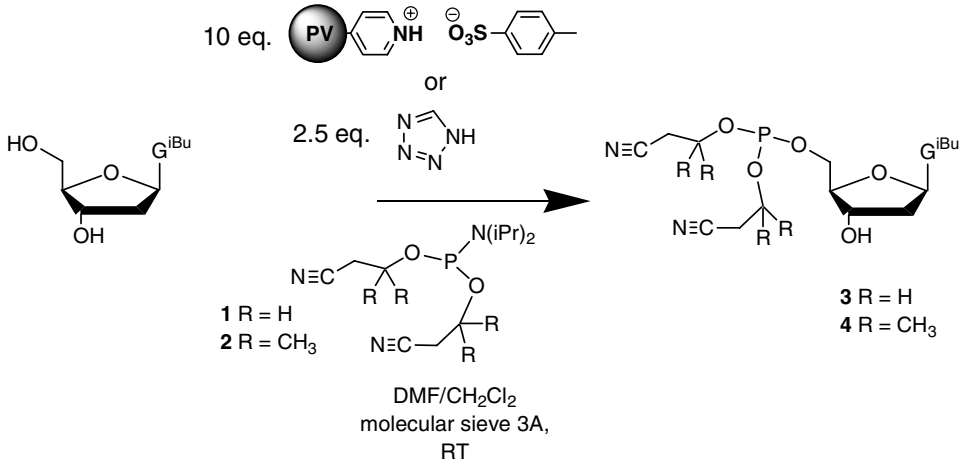
The nucleoside was dissolved in dichloromethane/DMF (1:1, v/v) and dried overnight with 3 Å molecular sieve. The coupling agent PVPtos (10 M equiv) or tetrazole (2.5 M equiv) was then added followed by phosphine **1** or **2**, added as a 0.3 M solution in CH_2Cl_2 in three parts (i.e., for **1**: 0.60, 0.45, and 0.30 M equiv; for **2**: 1.05, 0.30, 0.15 M equiv) at room temperature. The reaction progress was monitored by HPLC. The percentage of each phosphitylated species was calculated and is shown in Table 1 with the percentage of conversion of the starting $dG^{i\text{-Bu}}$ nucleoside.

These data confirm that classic conditions using phosphine **1** and tetrazole led to a moderate 5′-selectivity. During the course of the reaction, a 84% selectivity was obtained when 83% of $dG^{i\text{-Bu}}$ conversion was reached. Unfortunately, the addition of phosphine to drive the reaction up to 90% was damaging since only

Table 1. Regioselective phosphitylation of N^2-isobutyryl-2′-deoxyguanosine with tetrazole or PVPtos as activator and phosphine **1** or **2**

Entry	Phosphine activator	Equiv of phosphite (**1** or **2**)[a]	Ratio of phosphites (%)			Conversion (%)[b]
			5′-	3′-	3′,5′-	
1	**1** Tet.	0.60 (0.5)	76	14	10	46
		1.05 (0.93)	84	4	12	83
		1.35 (1.07)	70	3	27	90
2	**2** Tet.	1.05 (0.55)	92	5	3	53
		1.35 (0.71)	91	5	4	68
		1.50 (1.02)	86	5	9	89
3	**1** PVPtos	0.60 (0.32)	89	9	2	32
		1.05 (0.91)	91	6.5	2.5	90
		1.35 (1.01)	90	5	5	98
4	**2** PVPtos	1.05 (0.45)	96	3	1	45
		1.35 (0.66)	96	2	2	65
		1.50 (0.93)	95	2	3	91

[a] In the parenthesis, the effective equivalent of phosphitylating agent consumed.
[b] Percentage of $dG^{i\text{-Bu}}$ consumed.



Scheme 1. Selective 5′-phosphitylation of N^2-isobutyryl-2′-deoxyguanosine.

70% of the desired compound **3** was formed with 27% of 3′,5′-diphosphitylated nucleoside (Table 1, entry 1). Interestingly, the use of the bulky phosphine **2** greatly improved the selectivity with 86% of 5′-phosphitylated nucleoside **4** formed and only 9% of diphosphitylated one, keeping a similar conversion (89%) of $dG^{i\text{-}Bu}$ (Table 1, entry 2).

The use of a heterogeneous activation by means of PVPtos gave a high 5′-regioselectivity with 90% of the desired compound **3** formed with phosphine **1** with almost quantitative conversion of starting $dG^{i\text{-}Bu}$ (Table 1, entry 3). As previously, the phosphitylation by means of phosphine **2** improved the regioselectivity to 95% (Table 1, entry 4). For the both phosphines, only few amounts of 3′,5′-diphosphitylated (3–5%) and 3′-phosphitylated (2–5%) nucleosides were formed (Table 1, entries 3 and 4). These results are extremely interesting since the heterogeneous activation allowed a high 5′-regioselectivity (90–95%) in combination with a high conversion (98–91%) of the starting nucleoside.

These data clearly demonstrate that (1) with a standard tetrazole activation, the use of a bulky phosphine increases the selectivity from 70% to 86% with 90% of conversion, (2) the use of a heterogeneous activation by means of a solid-supported activator improved the selectivity (70–90%) with a quasi-quantitative conversion of the starting $dG^{i\text{-}Bu}$, (3) the combination of a heterogeneous activation and a bulky phosphine gave the highest selectivity (95%).

The 5′-phosphitylation of N^2-isobutyryl-2′-*O*-methyl guanosine was then performed by means of PVPtos as an activator with either phosphine **1** or **2** added, as a 0.3 M solution in CH_2Cl_2, in three times 0.60, 0.45, and 0.30 M equiv (Scheme 2 and Table 2). Between each addition, the progress of the reaction was monitored by HPLC. No significant difference of reactivity was observed between both phosphines.

The data clearly demonstrated a high selectivity of 5′-phosphitylation 88% and 95% using phosphines **1** and **2**, respectively, with an almost quantitative conversion of starting material (96% and 100%). This latter point is particularly important because usually when the conversion increases the selectivity decreases.[2]

After completion of the phosphitylation, the mixture was filtered off to remove the solid-supported coupling agent and the resin was washed with CH_2Cl_2. After concentration to half-volume, the phosphite triester linkage was sulfurized into thionophosphorotriester using solid-supported tetrathionate.[10] The conversion of **5** into **7** was completed after an overnight treatment although it took 3 days for the conversion of **6** into **8** (HPLC monitoring). This latter result could be explained by the higher steric hindrance due to the four extra methyl groups in β,β′ of the phosphorous atom. After filtration and evaporation, the desired compounds were isolated pure by flash chromatography on silica gel with a good yield (**7**: 70% and **8**: 80%). Alternatively, compound **5** was oxidized into phosphotriester by means of the solid-supported periodate[10] within 2 h and was isolated after chromatography (75%). At this stage, the 5′-*O*-thionophosphotriester or 5′-*O*-phosphotriester nucleosides could be either deprotected by ammonia to afford the corresponding 5′-*O*-mono(thio)phosphate nucleosides or could be used for a further coupling to afford 5′-(thio)phosphorylated dimers. Along this line we used **7** and **8** for a coupling with 5′-*H*-phosphonate *N*,3′-*O*-*acyl* nucleosides to afford dimers. This work will be reported elsewhere.

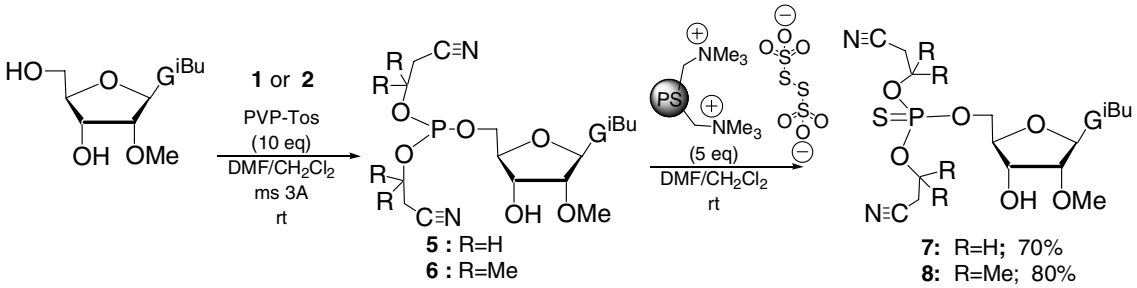
Table 2. Regioselective phosphitylation of N^2-isobutyryl-2′-*O*-methylguanosine with PVPtos as activator and phosphine **1** or **2**

Phosphine	Equiv of phosphite (**1** or **2**)[a]	Ratio of phosphites (%)			Conversion (%)[b]
		5′-	3′-	3′,5′-	
1	0.6 (0.48)	95	2.5	2.5	47
	1.05 (0.89)	93	1.5	5.5	85
	1.35 (1.07)	88	0	12	96
2	0.6 (0.52)	98	1.4	0.6	52
	1.05 (0.88)	97	1	2	87
	1.35 (1.03)	95	0	5	100

[a] In the parenthesis, the effective equivalent of phosphitylating agent consumed.

[b] Percentage of 2′-*O*-Me-$G^{i\text{-}Bu}$ consumed.

In conclusion, we have developed a highly regioselective phosphitylation of N-protected guanosine derivatives using a heterogeneous activation by means of a solid-supported activator (PVPtos). Maximal regioselectivity was obtained using a bulky phosphine in combination. Then, their conversion into (oxo and thiono) phosphotriesters was also performed with solid-supported oxidizers. The great advantage in using solid-supported reagents is that all the work-ups and purifications are



Scheme 2. Selective 5′-phosphitylation of N^2-isobutyryl 2′-*O*-methyl guanosine and its sulfurization.

easier since a simple filtration stops the reaction and removes the excess of reagents. Furthermore, these solid-supported reagents are cheap and easily reusable.

We expect that this method would be extended to the preparation of other 5′-phosphate nucleoside analogs.

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Supplementary data

Supplementary data (the protocols for the synthesis of **2** and for phosphitylation and oxidation to afford **7** and **8** with NMR and MS data) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.09.086.

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