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COMMUNICATION

Microwave-Assisted Preparation of Nucleoside-Phosphoramidites

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Microwave-assisted phosphitylation of sterically hindered nucleosides is demonstrated to be an efficient method for the preparation of corresponding phosphoramidites (otherwise onerous under standard conditions) and shown to be general in its applicability.

Nucleoside-phosphoramidites are ubiquitous and in high demand owing to their use in oligonucleotide synthesis.^{1,2} Research into modified oligonucleotides have intensified due to their potential in therapeutic applications. The preparation of modified nucleoside phosphoramidites³ has been accomplished by the extension of protocols developed in the canonical nucleic acid series and the various commercially available reagents.⁴

We present here details of microwave-assisted phosphitylation of sterically hindered ribulose nucleosides (Figure 1), using commercially available phosphitylating reagents. This methodology is shown to be general in its nature and is further illustrated by the preparation of phosphoramidites in the DNA and RNA series, suggesting that contrary to the widely held belief, nucleoside-phosphoramidites could be prepared and remain stable under elevated temperature conditions.⁵

For the purpose of investigating the base-pairing properties of pentulose derived nucleic acids,⁶ in the context of the chemical etiology of nucleic acid structure⁷, we required the 3'-*O*-phosphoramidites of the ribulothymidine and ribuloadenine

nucleosides (Figure 1). However, the use of standard phosphitylation protocols afforded consistently low yields of the crucial phosphoramidites, with inefficient conversion of starting materials (Scheme 1). For example, the phosphitylation reaction of **3** using 2-cyanoethyl *N,N,N'*-diisopropylchlorophosphoramidite **5** afforded **7** in only 35% yield (Table S1, entry 1). We ascertained that phosphoramidite **7** was stable and that the low yields were because of an inefficient reaction. Changing the reaction time or the base did not produce any improvements (Table S1, entries 2 and 3). Switching to the alternative reagent 2-cyanoethyl-*N,N,N',N'*-tetraisopropyl phosphane **6** and exploring various activators (Scheme 1), resulted in lower yields of the desired product (Table S1, entries 4–8).

In addition to the benzoylated ribulose nucleoside **3**, we also prepared the 4'-*O*-TOM-protected ribulo-thymidine derivative **4** and the corresponding 4'-*O*-TOM-protected ribulo-adenine derivative **9**.⁶ Phosphitylation of **4** and **9** using the standard reaction conditions (table S1), was again inefficient producing low amounts of phosphoramidites **8** and **12** respectively.

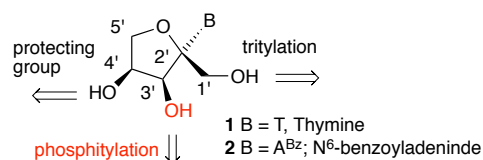
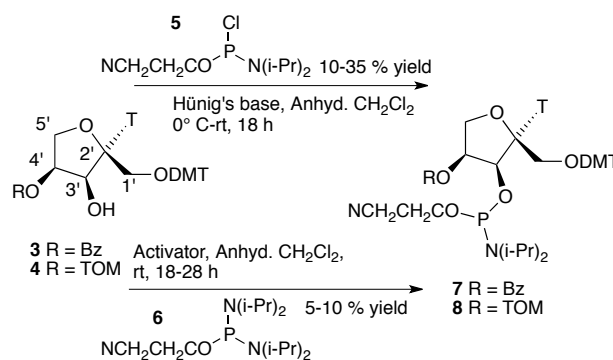


Figure 1 (L)-Ribulose-derived nucleosides



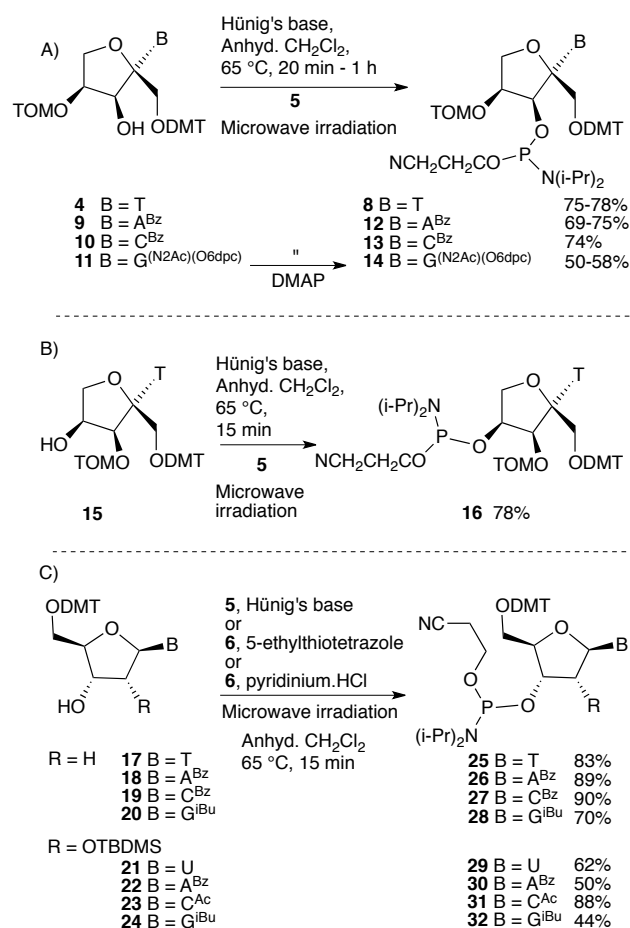
Scheme 1 Phosphitylation reactions under standard conditions. Bz = benzoyl; TOM = tri-iso-propylsilyloxymethyl.

Moreover, incomplete conversions led to purification problems; it became imperative to have the complete consumption and conversion of substrates **4** and **9** since we could not afford to lose precious material in this penultimate step before proceeding with oligonucleotide synthesis. While inspecting the reasons for the inefficient phosphorylation reaction, it became clear that the reaction outcome seemed (a) not affected by the nature of the phosphitylating agent used, and (b) independent of the nucleobase (purine or pyrimidine), and the type of protecting group at the C-4'-O-position. This indicated that possibly the equatorial disposition of the C-3'-OH group was exacerbating the intrinsic steric hindrance of the ribulo-nucleosides at C-3' (flanked by a quaternary C-2' and tertiary C-4'). This might result in the low nucleophilicity of the 3'-OH group, and the inefficient phosphorylation reaction.[†] Such an 'inherent structural' limitation could be overcome by using a less sterically hindered phosphitylating agent such as a diethylamino derivative of either **5** or **6**.^{8,9} However, the increased instability of these reagents coupled with their commercial non-availability discouraged us from pursuing this option. Another recourse was to increase the temperature of the phosphorylation reactions; however, based on the widely perceived 'instability of phosphoramidites' when exposed to high temperatures,⁵ we did not take this route.

The recent use of ionic liquids as solvents coupled with mechanochemistry to perform phosphorylation of selected canonical nucleosides⁹ encouraged us to consider unconventional experimental procedures. We decided to explore the potential of microwave assistance based on the demonstration that many inefficient processes were rendered productive by microwave irradiation.¹⁰ Although, unsure about the stability of the 'sensitive' phosphoramidites under the microwave-assisted conditions we decided to explore the microwave option out of sheer need for phosphoramidites of the ribulose nucleosides for our studies. The reaction of nucleoside **4** with phosphitylating reagent **5** under microwave-assisted conditions was investigated, in anhydrous CH₂Cl₂ in the presence of Hünig's base with 2 equivalents of phosphitylating reagent **5** for 1 h (Scheme 2A). We were pleasantly surprised to observe clean conversion to the product. The ³¹P NMR spectrum of the crude mixture showed major peaks at 152.7 and 152.4 ppm, with minor peaks indicative of side reactions (Figure S6a). The crude product was directly purified by column chromatography to isolate 75% of pure phosphoramidite **8** (Figure S6b). The reaction time was optimized and 20 min was found to be sufficient for the reaction to proceed to completion. The sequence of addition of (the reagent and substrate) seems not be important. When a solution of compound **4** in anhydrous CH₂Cl₂ together with Hünig's base was added to 2 equivalents of phosphitylating reagent **8** in a microwave tube and irradiated at 65 °C for 30 min, it resulted in the complete consumption of starting material. It was gratifying to observe that phosphitylated compound **8** was produced efficiently and that it was stable under microwave-assisted-synthetic conditions. The application of the microwave-assisted procedure to the ribulo-adenosine

derivative **9** and ribulo-cytidine derivative **10** afforded good yields of the corresponding phosphoramidite derivatives **12** and **13**, respectively. For the guanine derivative **11**, the optimized microwave conditions, surprisingly, led to no product formation; however, with addition of DMAP, the microwave assisted reaction proceeded cleanly to afford **14**. The difficulty experienced with substrate **11** may be related to the presence of the N²(Ac)-group on the guanine that could lead to more steric hindrance for reaction at the 3'-OH position.[#]

Two points of this microwave-assisted reaction are noteworthy: (1) the temperature of the reaction mixture was at around 65 °C (with CH₂Cl₂ as the solvent). The fact that phosphoramidite products were stable under the higher-than-conventional temperatures (0 °C to rt) was unanticipated, and (2) the sterically hindered nucleosides were completely consumed, facilitating easy chromatographic purification.



Scheme 2 Phosphitylation reactions with microwave assistance.

The above results demonstrated that the microwave-assisted phosphorylation could be an efficient process, particularly suited for the production of hindered phosphoramidites. This was reinforced by the outcomes from attempts to phosphitylate 3'-O-TOM derivative **15** (a regioisomer of **4**). The phosphorylation

reaction on regioisomer **15** under standard conditions with reagent **5** for 5 h at rt led to an incomplete reaction with derivative **16** isolated in 40% yield (containing 4% of starting material). In contrast, when nucleoside **15** was phosphitylated under microwave conditions (at 65 °C) for 15 min, the yield of compound **16** nearly doubled to 78% (Scheme 2B).

Having demonstrated that microwave-assisted phosphitylation succeeded with sterically congested ribulose-nucleosides, we decided to probe the general applicability of this procedure for the preparation of DNA and RNA phosphoramidites (Scheme 2C). The aim was to check whether DNA and RNA-nucleoside-phosphoramidites could be produced under microwave conditions given the concerns for their sensitivity⁵ as ‘fragile’ nucleoside-phosphoramidites. The microwave-mediated phosphitylation reaction was tested on suitably protected 2'-deoxyadenosine **18** using reagent **5** and the corresponding phosphitylated derivative **26** was isolated in 75% yield (Table S2, entry 1). Since there is a preference for the 2-cyanoethyl-*N,N,N,N'*-tetraisopropyl phosphoramidite **6** as a phosphitylating reagent owing to its stability against hydrolysis when compared to **5**,^{1,2} we also investigated microwave-assisted-phosphitylations of commercially available DNA and RNA substrates employing **5** with Hünig's base and **6** with 5-ethylthiotetrazole or pyridinium hydrochloride as activators (Scheme 2C). Good to excellent yields (70–90%) of phosphoramidites **25–28** were obtained (Table S2, entries 1–6). In the case for RNA phosphoramidites **29**, **30**, and **32**, the yields were lower (40–62%) and in the case of **32**, substantial amounts of H-phosphonate by-products were formed indicating that further optimizations are needed.[†] However, the stability of the phosphoramidites formed under microwave reaction conditions is noteworthy.

Conclusions

Phosphitylation of sterically hindered nucleosides (which was problematic under standard conditions) with commercially available reagents was rendered efficient by microwave-irradiation. The resultant phosphoramidites were formed within short time spans (15–20 min.) and found to be stable under the reaction conditions. This suggests that phosphitylation reactions need not be restricted to mild conditions and could have more flexibility with respect to reaction parameters (e.g. higher temperature). This microwave-assisted phosphitylation reaction has been shown to be general in its nature for nucleosides, with some optimizations needed in the RNA series. It is proposed that the microwave-assisted phosphitylation procedure outlined here could become a useful tool with the potential to accommodate a wide variety of substrates.

Notes and references

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¶ TOM-derivatives were prepared based on protecting group compatibility considerations (originating from the difficulties encountered in oligonucleotide synthesis using the phosphoramidites with the 4'-O-benzoyl group).

† Consistent with this reasoning the corresponding xylulo-derived nucleosides –where the 3'-OH group is axial (but have the same protecting groups at C-4' and C-2')– were phosphitylated under the standard reaction conditions with phosphoramidite yields ranging from 78–90%.⁶

This is inferred from the x-ray structure of the ribulo-adenine nucleoside that places the N(3) of the purine ring in close proximity to the 3'-OH group.⁶

+ The lower (unoptimized) yields may be reflective of the difference in the TOM vs. TBDMS protecting groups.

Electronic Supplementary Information (ESI) available: Experimental procedures and spectral characterization of compounds; Figures S6a and S6b, Tables S1 and S2. See DOI: 10.1039/c000000x/

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