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Microwave-assisted preparation of nucleoside-phosphoramidites†

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G. Meher, T. Efthymiou, M. Stoop and R. Krishnamurthy*

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 is shown to be general in its applicability.

Nucleoside-phosphoramidites are ubiquitous and are in high demand owing to their use in oligonucleotide synthesis.[1,2] Investigation of modified oligonucleotides has intensified due to their potential in therapeutic applications. The preparation of modified nucleoside phosphoramidites[3] 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 the details of microwave-assisted phosphitylation of sterically hindered ribulose nucleosides (Fig. 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 structure,[7] we required the 3′-*O*-phosphoramidites of the ribulo-thymidine and ribulo-adenine nucleosides (Fig. 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*,-diisopropylchlorophosphoramidite **5** afforded **7** in only 35% yield (Table S1, entry 1, ESI†). 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 show any improvement (Table S1, entries 2 and 3, ESI†). Switching to the alternative reagent 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl phosphorodiamidite **6** and exploring various activators (Scheme 1), resulted in lower yields of the desired product (Table S1, entries 4–8, ESI†).

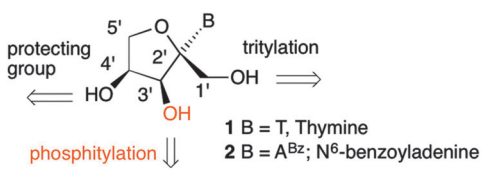
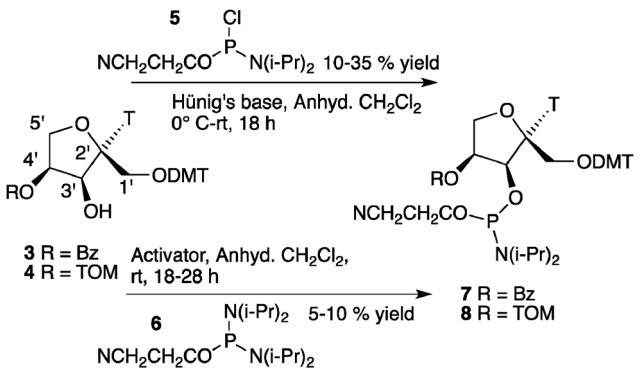


Fig. 1 (L)-Ribulose-derived nucleosides.



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

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, ESI†), was again inefficient, producing low amounts of phosphoramidites **8** and **12** respectively.

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

Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA. E-mail: rkrishna@scripps.edu

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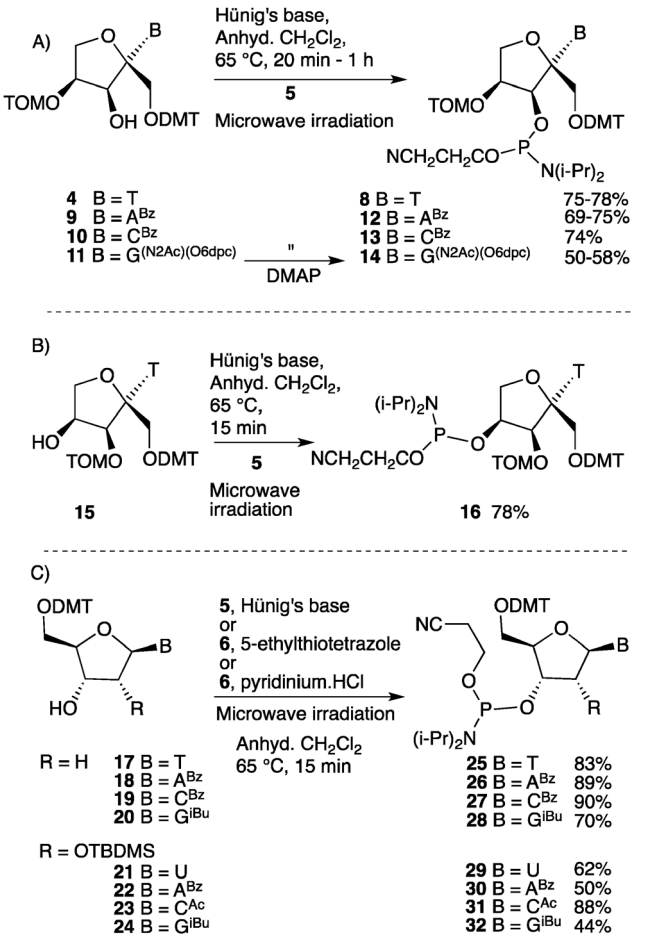
the inefficient phosphitylation reaction, it became clear that the reaction outcome seemed to be (a) not affected by the nature of the phosphitylating agent used, and (b) independent of the nucleobase (purine or pyrimidine), and the type of protecting group at the C-4′-*O*-position. This indicated that 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 phosphitylation 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 phosphitylation 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 phosphitylation of selected canonical nucleosides[9] 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 CH_2Cl_2 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 ^{31}P NMR spectrum of the crude mixture showed major peaks at 152.7 and 152.4 ppm, with minor peaks indicative of side reactions (Fig. S6a, ESI†). The crude product was directly purified using column chromatography to isolate 75% of pure phosphoramidite **8** (Fig. S6b, ESI†). 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 the substrate) seems to be not important. For example, when a solution of compound **4** in anhydrous CH_2Cl_2 together with Hünig's base was added to 2 equivalents of the 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 the 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, upon 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 N^2(Ac)-group on the guanine that could lead to more steric hindrance for reaction at the 3′-OH position.¶



Scheme 2 Phosphitylation reactions with microwave assistance.

Two points of this microwave-assisted reaction are noteworthy: (1) the temperature of the reaction mixture was at around 65 °C (with CH_2Cl_2 as the solvent). The fact that phosphoramidites 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.

The above results demonstrated that the microwave-assisted phosphitylation could be an efficient process, particularly suited for the production of hindered phosphoramidites. This was reinforced by the outcomes from attempts to phosphitylate the 3′-*O*-TOM derivative **15** (a regioisomer of **4**). The phosphitylation reaction of 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 the 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 sensitivity[5] 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, ESI†). Since there is a preference for the 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl phosphorodiamidite **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 DNA phosphoramidites **25–28** were obtained (Table S2, entries 1–6, ESI†). In the case of RNA phosphoramidites **29**, **30**, and **32**, the yields were lower (44–62%) 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.

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.

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Notes and references

‡ 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 to 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.

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