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COMMUNICATION

Nucleoside phosphitylation using ionic liquid stabilised phosphorodiamidites and mechanochemistry†‡

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A range of nucleoside phosphoramidites incorporating small amino substituents have been readily synthesised using ionic liquid stabilised phosphorodiamidites coupled with mechanochemistry.

Within the field of carbohydrate and nucleotide chemistry, novel methods are currently being widely explored for the derivatisation of sugar moieties where traditional methods are hampered by challenging syntheses which require multi-step processes and selective functional group manipulations.[1] In this regard, mechanochemistry has been used to overcome issues such as reagent solubility and reactivity,[2] while ionic liquids (ILs) have been shown to stabilise hydrolytically sensitive reagents.[3] Herein, we have combined these approaches to enable the phosphitylation of deoxyribo- and ribo-nucleosides using hydrolytically unstable phosphorodiamidite reagents in high yields. Furthermore, we have negated the need for the expensive activator 1*H*-tetrazole by using a laboratory-friendly alternative in the form of pyridinium trifluoroacetate (Py·TFA).

Nucleoside phosphoramidites are key building blocks for the automated, solid supported syntheses of oligonucleotide-based therapeutics. Many of these antisense oligonucleotide-based drugs are being evaluated for the treatment of a variety of disease states.[4] Variations at the phosphorus centre whereby small amino substituents are utilised to access chemically adaptable nucleoside phosphoramidites are limited[5] despite the notion that they improve the rates of the coupling process and increase oligomerisation yields. For example, Chamberlain has demonstrated that in automated RNA oligonucleotide synthesis, nucleoside phosphoramidites incorporating small amines were more readily oligomerised.[6] However, access to oligonucleotides *via* these small amino derivatives is more challenging due to hydrolytic instability of both the phosphitylation reagents as well as the resulting nucleoside phosphoramidite building blocks. Although bis(dialkylamino)methoxyphosphines, $P(OMe)(NR_2)_2$, which incorporate small amino functionalities have been used for the rapid preparation of deoxynucleoside phosphoramidites, the methodology is not straightforward.[7] Firstly, the method requires strictly anhydrous conditions with respect to both reagents and glassware. Secondly, the nucleoside phosphoramidites are prepared *in situ* and are not isolated. These were, however, shown to provide an efficient route towards oligonucleotides. Thus, in order to provide a balance between hydrolytic stability and to allow for effective oligomerisation, bulky diisopropylamino derivatives are the preferred reagents.[8] 2-Cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite has been used in this regard; however, this reagent is expensive, moisture sensitive and highly unstable at room temperature.[9] Consequently, 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite is commonly used as an alternative reagent in conjunction with a proton-source activator.[10] Although this provides a route to access relatively stable nucleoside phosphoramidites, the presence of the diisopropylamino groups means that their chemical reactivity is reduced by virtue of steric hindrance.[11] Despite extensive optimisation of the current solution-based methodologies for the effective syntheses of deoxy- and ribonucleoside phosphoramidites, the procedures still suffer from a number of issues including low yield and low stability of the resulting nucleoside phosphoramidites. These issues can be associated with the poor solubility of the nucleosides in apolar organic solvents. This makes it necessary to conduct the phosphitylation reactions in polar solvents such as dichloromethane (DCM), pyridine or dimethylformamide (DMF). However, the use of polar solvents increases the probability of hydrolysis of the reagents or products due to the increased hydrophilicity of the solvents.

The use of phosphorodiamidite reagents require the use of a weakly acidic *in situ* activator to allow for activation of the P–N bond. 1*H*-Tetrazole is the most widely used activator for this transformation; yet it possesses a number of practical issues. Firstly, 1*H*-tetrazole is a classified explosive and highly toxic.[12] Secondly, 1*H*-tetrazole is expensive and, therefore, the overall phosphitylation process is not cost effective.[13]

ILs have been shown to provide a unique medium for the synthesis, stabilisation and reactivity of a range of hydrolytically unstable phosphorus reagents, including chlorophosphoramidites,[14] while mechanochemistry is being used increasingly for a wide array of organic transformations.[15] This solvent free technique has been applied to enable nucleoside silylation in one pot using solvent-free ball milling conditions.[16] More recently, it was demonstrated

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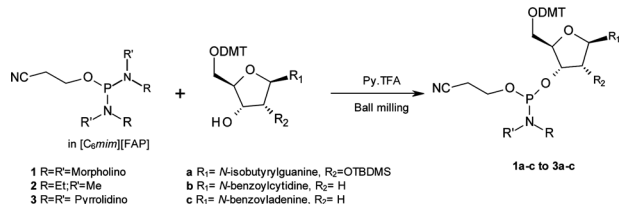

Fig. 1 Schematic representation of the reaction of phosphorodiamidites to form nucleoside phosphoramidites using mechanochemistry and a stabilising IL.

that IL stabilised chlorophosphoramidites in conjunction with ball milling, mediated the successful phosphitylation of nucleosides and 2-deoxynucleosides even when the chlorophosphoramidite incorporated small amino functionalities.[14c] Herein, we show that phosphorodiamidites in the presence of a stabilising IL environment and a weakly acidic activator could be ball-milled with both deoxyribo- and ribo-nucleosides leading to the production of phosphitylated nucleosides in high yields.

The phosphorodiamidites used, herein, (**1–3**, Fig. 1) were prepared according to the IL procedure previously reported.[17] In this instance, they were extracted into a small amount of 1-hexyl, 3-methyl imidazolium tris(pentafluoroethyl)trifluorophosphate ([C_6mim][FAP]) which acted as a stabiliser prior to concentration. In this case, the [C_6mim][FAP] and phosphorodiamidite were present as a 1 : 1 mole ratio. Although ILs with smaller imidazolium alkyl chain lengths (ethyl or butyl) allow for successful phosphitylation, the reduced interaction of the phosphorus reagent with the smaller alkyl chain means hydrolytic stability is compromised.[14c] Hence, in this study [C_6mim][FAP] was used for all the reactions undertaken.

Using the [C_6mim][FAP]-stabilised phosphorodiamidites, the ball milled reactions were performed using the mechanochemical procedure outlined by Norman *et al.*[14c] However, in this instance an *in situ* activator was used instead of the Hünig's base. The yields of **1a** from the IL-stabilised bis-morpholino phosphorodiamidite, **1**, with a range of activators are summarised in Table 1. Although **1a** was obtained in high yield (80%) using 1*H*-tetrazole, a replacement for this hazardous substance was explored as the explosive nature of this material means it would not be suitable for ball milling at larger scales. Both *N*-methylimidazolium triflate (NMI·OTf) and Py·TFA are readily prepared from the corresponding acid and have been shown to be effective, cheap alternatives to 1*H*-tetrazole.[18] Using Py·TFA, **1a** could be isolated in a yield of 85% after purification. The purification was performed simply *via* filtration through silica thus negating the need for extensive and time consuming workup procedures. The same work-up procedure was used when NMI·OTf was used as the activator, but as Py·TFA resulted in slightly enhanced yields it was used in all subsequent reactions. Even though the use of NH_4Cl failed to yield **1a**, it is important to note that even after 30 min ball milling, only a small amount of degradation of **1** was observed by ^{31}P NMR, thus highlighting the stabilising nature of the IL. However, due to the high hygroscopic nature of the other activators investigated, an optimal 2 : 1 mole ratio of phosphorodiamidite : nucleoside was implemented. This was to ensure that reaction completion was not compromised by degradation of the starting phosphorodiamidite due to the activator-promoted water content of the reaction mixture over time. Even after drying the activator under high vacuum, it was difficult to remove all traces of water as evidenced by the presence of a small amount of decomposed phosphorodiamidite in the crude reaction mixture. This observation was complemented by Karl-Fischer data obtained before and after drying of the activator. For example, prior to drying, the water content of Py·TFA was 10 wt%. After drying under vacuum this decreased to 1 wt%. In comparison, the water content of NH_4Cl was 0.3 wt% even prior to drying. Despite the high water contents, the reactions were high yielding and clean with only the product and easily separable hydrolysed phosphorodiamidite present as shown by the NMR data of the crude mixtures (see ESI‡).

This mechanochemical approach was readily extended to include the phosphitylation of **1** with 2-deoxycytidine, **b**, and 2-deoxyadenosine, **c**, in addition to the IL-stabilised ethyl methyl phosphorodiamidite, **2**, with nucleosides **a–c** (Table 2). For all reactions performed, Py·TFA was the activator of choice. The isolated yields given in Table 2 are comparable to those previously reported under similar reaction conditions,[14c] but with greatly reduced reaction times and purification protocols compared with the solvent-based procedures of the diisopropylamino substrates. For all systems in Table 2, again a simple workup *via* filtration through silica could be applied. The simplicity of the overall procedure allows for the inclusion of small amino functionalities as well as eliminating the need for the traditionally implemented aqueous work-up. The co-existence of the *H*-phosphonate with the nucleoside phosphoramidites does not affect the purification of the product since it is inert and non-reactive. Furthermore removing the need for an aqueous work-up means a significant portion of the phosphitylated nucleoside is not lost *via* hydrolysis. This is particularly important as the hydrolytic instability of nucleoside phosphoramidites are well documented, particularly with respect to the smaller amino substituents.[18a] Importantly, in this case, the nucleoside phosphoramidites are isolated in a pure form which is a significant advantage over solvent based protocols where the nucleoside phosphoramidites must be generated *in situ*.[7] In addition, unlike for the methoxy groups used in previously reported protocols for small amino-substituents,[7] the cyanoethyl group can be readily removed *via* β-elimination using a mild base. Due to the high hydrolytic instability of these materials, a stabilising amount of IL could be

Table 1 Comparison of the yields from the reaction of bis-morpholino phosphorodiamidite (**1**) with partially protected guanosine (**a**) using various activators

Entry	Activator	Yield (%)
1	NH_4Cl	0
2	1*H*-Tetrazole	80
3	NMI·OTf[a]	83
4	Py·TFA	85

[a] *N*-Methylimidazolium triflate.

Table 2 Yields for the nucleoside phosphitylation ball milling reactions using Py·TFA

Protected nucleoside	Amine	Yield (%)
Guanosine (**a**)	–N(Morpholino)$_2$ (**1**)	85
	–NEtMe (**2**)	80
2-Deoxy cytidine (**b**)	–N(Morpholino)$_2$ (**1**)	83
	–NEtMe (**2**)	74
2-Deoxy adenosine (**c**)	–N(Morph olino)$_2$ (**1**)	86
	–NEtMe (**2**)	75

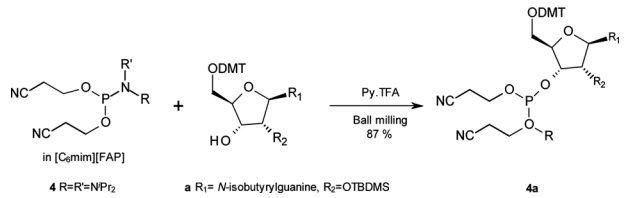

Fig. 2 Synthesis of **4a** from partially protected guanosine and phosphoramidite **4** using ball milling conditions.

added to allow for storage and further chemical manipulation. For example, in the presence of the IL, the ethyl methyl derivatives can be stored at low temperatures (−20 °C) for several months. In the absence of the IL, rapid degradation of the product occurs within a few hours.

The nucleoside phosphoramidites prepared, herein, are a mixture of diastereoisomers at the phosphorus centre, which is consistent for all commercially available nucleoside phosphoramidites. The isolated yields and diastereomeric ratios reported for **1a–2c** using our newly developed ball milling procedure are in good agreement with the solvent based protocols for phosphitylated nucleosides *via* the favoured diisopropylamino derivatives, where the yields range from 60–90% depending on the protected nucleoside used.[18a,19] The ball milling procedures documented, herein, have significant advantages over solvent based protocols including increased reaction rate taking minutes *vs.* hours for complete conversion, as well as improved reaction profiles.

In contrast to the dimorpholino and ethylmethyl derivatives, the dipyrrolidino derivative **3** was found to be highly unstable under the ball milling conditions used for the preparation of **1a–c** and **2a–c**. The phosphorodiamidite **3** rearranged rapidly to the corresponding alkylphosphonic diamide as shown by the peak at 26 ppm in the ^{31}P NMR spectra of the reaction's crude.[17] Interestingly, when the [C_6mim][FAP] : phosphorodiamidite mole ratio was increased to 2 : 1, successful phosphitylation by **3** was observed. In contrast to **1a–c** and **2a–c**, some decomposition of **3a** was evident by the ^{31}P NMR of the crude mixture. However, **3a** could still be isolated after purification in the presence of [C_6mim][FAP], albeit in much lower yields (15%) than for the other phosphorodiamidites studied.

The study has also been extended to examine the phosphitylation of partially protected guanosine, **a**, with bis-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite **4** in order to demonstrate the applicability of this newly developed ball-milling protocol to include phosphoramidites (Fig. 2). Phosphoramidites are used as phosphitylation reagents for a wide range of chemical transformations such as the derivatisation of alcohols[20] and peptides.[21] Using the reaction conditions developed, herein, the [C_6mim][FAP]-stabilised phosphoramidite **4** was used to phosphitylate partially protected guanosine **a** in high yields. Again, the reaction was clean and purification was performed *via* filtration through silica which allowed the isolation of **4a** in a yield of 87%.

The continued success of clinical programs incorporating oligonucleotide based therapeutics means that an environmentally benign, clean and cost efficient synthesis of these molecules is highly desirable. By combining ball milling with IL stabilisation of reactive phosphorus species, we have developed an efficient protocol to effect nucleoside phosphitylation with enhanced yields and reactivities. Overall, the method provides significant potential to extend the use for phosphitylation chemistry by increasing the ease of reaction and workup procedures.

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