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Overcoming hydrolytic sensitivity and low solubility of phosphitylation reagents by combining ionic liquids with mechanochemistry†

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Ionic liquids have been used in combination with ball milling on a range of chlorophosphoramidite reagents to phosphitylate nucleosides and 2-deoxynucleosides. The enhanced stability offered by the ionic liquid mediated processes combined with efficient mass transfer induced by ball milling has enabled excellent yields to be obtained even when using small dialkyl amino groups as well as the more commonly used diisopropylamino protection.

Two common obstacles to performing efficient chemical transformations are insolubility of reagents and decomposition of sensitive reagents/products. Ionic liquids (ILs), even when used as an additive rather than a solvent, can provide a protective medium in which hydrolytically sensitive species can be conveniently handled,[1] whilst mechanochemistry provides a way to perform reactions on poorly soluble species.[2] Herein, we demonstrate, for the first time, that the advantages of these two approaches may be combined to enable new chemistry. Specifically, we have combined these approaches to achieve enhanced reactivity, improved reaction profiles and increased yields in the phosphitylation of hindered secondary alcohols with a range of hydrolytically labile chlorophosphoramidites.

Oligonucleotides address therapeutic needs for a variety of disease states.[3] However, methods for phosphitylation of nucleosides (synthetic precursors to oligonucleotides) have not changed significantly since the 1980s.[4] This has limited the availability of reactive and functionally versatile nucleoside phosphoramidites, in particular for the preparation of the more structurally congested ribonucleic acid species. Although a range of alkoxy substituents have been examined, access to chemically adaptable nucleoside phosphoramidites using structurally diverse amino functionality has been restricted.[5] This lack of scope in nucleoside phosphoramidite building block availability is mainly due to the low stability of the phosphitylation reagents used for their preparation. To mitigate this limitation and provide means to access oligonucleotides which are sufficiently reactive and yet hydrolytically stable to allow for effective oligomerisation, bulky diisopropylamino derivatives are utilised in phosphoramidite nucleoside chemistry. These stable and commonly used diisopropylamino chlorophosphoramidite reagents have been optimised for use in DNA synthesis. In order to achieve oligomerisation yields comparable with that of DNA synthesis, RNA-synthetic chemists have manipulated the nature of the protecting groups at the C2′ position of the nucleoside phosphoramidites in order to increase reactivity and overcome steric hindrance which limits the phosphitylation process. However, an alternative, and potentially more versatile methodology, has been proposed and demonstrated by Chamberlin and co-workers.[5b] This method uses nucleoside phosphoramidites incorporating less hindered amino groups. Unfortunately, it is limited by the poor accessibility to its phosphitylating reagents. Although the presence of a less hindered amine at the phosphorus centre has been shown to enhance coupling rates, the reagents with smaller amines are more susceptible to decomposition. Considering the numerous emerging technologies and medicines relying on effective oligonucleic acid chemistry, development of more versatile reagents than those commonly in use would be timely. Current methods of synthesising ribonucleoside phosphoramidites have a number of issues including low yield, in terms of utilisation of the nucleoside *via* the phosphodiamidites, and low stability of the ribonucleoside phosphoramidites.

ILs have recently shown some success in mediating the phosphitylation of nucleosides using the N^iPr_2-stabilised reagent.[6] However, we show here that, in the presence of a stabilising IL environment, mechanochemical reaction conditions (ball milling) can also lead to high yields of dialkylamino phosphitylated derivatives of deoxyribo- and ribo-nucleosides (Fig. 1). In these reactions, the ionic liquid was present as 1.5 mol equivalent to the nucleoside and chlorophosphoramidite, *i.e.* the IL was an additive rather than a bulk solvent.

The chlorophosphoramidites (**1–3**, Fig. 1) used in this work were prepared in high yield using the IL process previously reported.[6] In each case, these chlorophosphoramidites could be stored in an IL in air for months without detectable degradation. Using the [C_6mim][FAP]-stabilised chlorophosphoramidites, the phosphitylations of partially protected nucleosides were attempted by adding dichloromethane (DCM) to solubilise the

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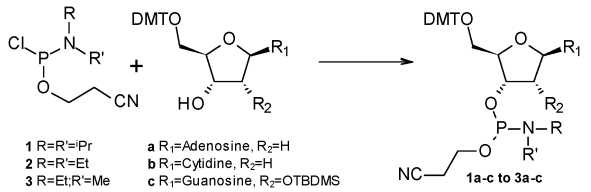

Fig. 1 Schematic showing the reaction of chlorophosphoramidites to form phosphitylated nucleosides using IL media. For synthetic details see ESI.†

partially protected nucleoside and Hünig's base with stirring. It should be noted that, without DCM, there was less than 5% conversion after 24 h. The diisopropyl amino derivatives showed excellent conversion with isolated yields of 84–92% for the 2-deoxy-ribose based nucleosides but only 46% conversion for the ribose parent, even when catalysed by the addition of 4-dimethylaminopyridine (DMAP). More surprisingly, only low yields were obtained using diethylamino and methylethyl amino chlorophosphoramidites. **2a** was only obtained in 15% yield after several days and still lower yields (<1%) were found for the methylethyl amino derivative (**3a**). This is unexpected because reactivity of the chlorophosphoramidite commonly increases on decreasing the size of the amine substituent.[5a] Furthermore, neither the addition of DMAP nor higher temperatures significantly improved the yield. In all cases, the main species found in the reaction mixture was the unreacted phosphitylation reagent, with <10% decomposition. Given the generally high reactivity of chlorophosphoramidites, the kinetics are thought to be mass transfer limited. This is supported by the fact that low concentrations of <50 mM for the protected nucleosides have been reported in $[NTf_2]^-$ based ILs and <10 mM in $[FAP]^-$ based ILs.[7] Clearly, good contact between the chlorophosphoramidite and the nucleoside must occur for high yields to be obtained and this is difficult to achieve due to the low solubility of the nucleoside in the IL phase.

The unusual reactivity trend (**1**) ≫ (**2**) > (**3**) observed under these DCM-based conditions is consistent with the qualitative visual observation that (**1**) has a significantly lower solubility in the ILs than either (**2**) or (**3**). Liquid mixtures often have complex heterogeneous structures on the molecular scale[8] and, therefore, it is possible that the IL–chlorophosphoramidite interaction is maintained even in DCM, thus decreasing the concentration of free, reactive phosphitylating reagents. In this case, as the solubility of the nucleoside in the IL is very low, its interaction with **2** or **3** will be significantly reduced compared with **1**, thereby decreasing the reaction rate. In order to vary the strength of IL interaction with **2**, a range of $[FAP]^-$ based ILs with differing imidazolium alkyl chain lengths (ethyl, butyl and hexyl) was investigated. Yields of **2a** from the IL stabilised diethylamino chlorophosphoramidite (**2**) under the standard reaction conditions (DCM, Hünig's base, stirring) are given in Table 1. As expected, and following from the observation that the solubility of **2** in the IL decreases as the IL alkyl chain length is shortened, the reaction yield was correspondingly greater with shorter IL alkyl chains (ethyl > butyl > hexyl). However, as expected, the reduced interaction of the phosphorus reagent with the shorter alkyl chain length based ILs also increased its hydrolytic sensitivity as shown by the presence of decomposition products in ^{31}P NMR spectra.

Table 1 Comparision of the yields from the reaction of diethylamino chlorophosphoramidite (**2**) with 2-deoxy adenosine (**a**) under various reaction conditions

Ionic liquid	DCM (24 h)	Sonication (30 min)	Ball milling (30 min)
[C_2mim][FAP]	65%	Decomposition of **2**	Decomposition of **2**
[C_4mim][FAP]	40%	40%	65%
[C_6mim][FAP]	15%	48%	82%

In order to improve the dissolution of the nucleosides in the IL, sonication and ball milling were investigated in the absence of DCM using the IL stabilised chlorophosphoramidite (Table 1). The ball milled reactions were performed using the procedure outlined recently for mechanochemical nucleoside silylation by Giri *et al.*[9] Remarkably, using ball milling, in the presence of only very small amounts of [C_4mim][FAP] and [C_6mim][FAP] as stabilisers (1.5 : 1 mole ratio IL : chlorophosphoramidite), much higher reaction rates and product yields resulted than under stirring or sonication. After 30 min, isolated yields of 40 to 82% were obtained with little decomposition (compared with <40% isolated yield for standard stirring after 24 h). For all reactions, the order of reagent addition was critical, with the best yields obtained when the chlorophosphoramidite was added to the Hünig's base and nucleoside mixture. If the base was not present before chlorophosphoramidite addition, cleavage of the DMT protecting group was observed. The most promising results occurred using the [C_6mim][FAP] stabilised materials under ball milling conditions. The overall yields were high and the reactions were clean with only the product and excess chlorophosphoramidite being detected in the crude mixture. Furthermore, during the reaction of **2a** and **2b**, no unreacted nucleosides were found by ^1H NMR even in the crude product.

The ball milled reactions were pastes, *i.e.* viscous liquid phases (primarily the IL, chlorophosphoramidite and Hünig's base) containing solid particles (primarily the nucleoside reagent and/or product). These reactions can be thought of as unusual liquid-assisted grinding (LAG) reactions[2d,10] in which the liquid may accelerate the reaction by aiding transport as well as provide a protective medium for sensitive species. This approach, as well as LAG with ILs, appears to be novel in mechanochemistry.

The mechanochemically promoted phosphitylation in [C_6mim][FAP] was readily extended to the other IL-stabilised diethyl and methylethyl chlorophoramidites as well as 2-deoxy-cytidine and guanosine nucleosides (**b** and **c**) (Table 2). All reactions under mechanochemical conditions gave high isolated yields (73–91%) compared with stirring in DCM where

Table 2 Yields for the nucleoside phosphitylation reactions for the DCM and ball milled reactions

Protected nucleoside	Amine	DCM (%)	Ball mill (%)
2-Deoxy adenosine (**a**)	–N^{i}Pr$_2$ (**1**)	92	80
	–NEt$_2$ (**2**)	15	82
	–NEtMe (**3**)	0	79
2-Deoxy cytidine (**b**)	–N^{i}Pr$_2$ (**1**)	84	80
	–NEt$_2$ (**2**)	9	91
	–NEtMe (**3**)	5	74
Guanosine (**c**)	–N^{i}Pr$_2$ (**1**)	46[a]	89
	–NEt$_2$ (**2**)	8	82
	–NEtMe (**3**)	5	73

[a] Addition of DMAP to the reaction mixture.

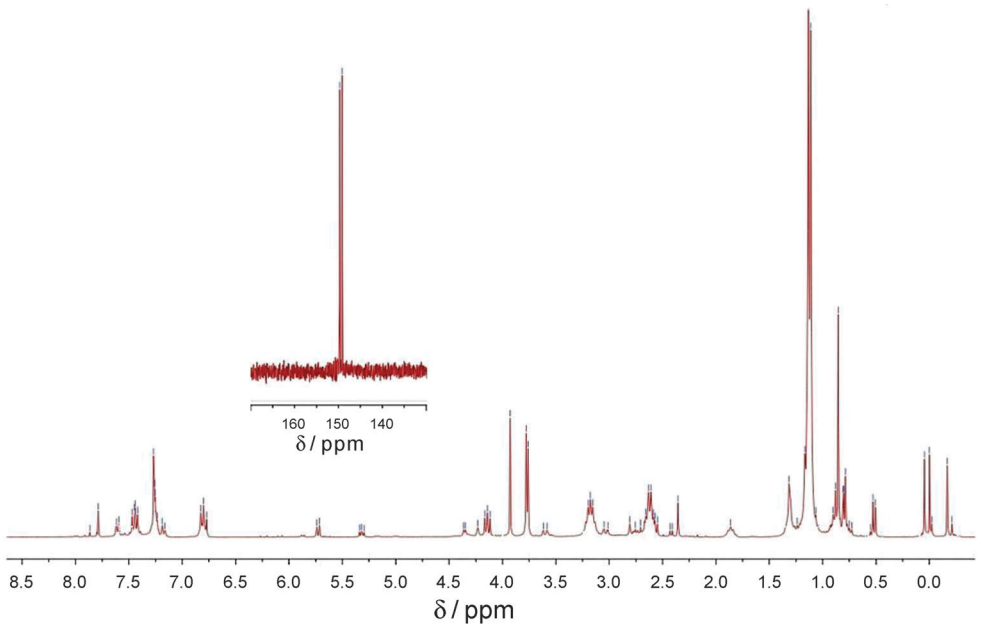

Fig. 2 ^{1}H NMR spectrum of compound **3c** after purification. The corresponding ^{31}P NMR spectrum is shown as the inset.

isolated yields ranged between 0 and 15% for the IL-stabilised diethyl and methylethyl chlorophoramidites. Remarkably, ^{31}P NMR revealed only the product and unreacted chlorophosphoramidite in all cases. In addition, simple workup by filtration through silica could also be applied, thereby, avoiding the need for aqueous workup and extensive purification methods (Fig. 2). For all systems, the nucleoside phosphoramidite was isolated in pure form and, for the more hydrolytically unstable derivatives, a stabilising amount of IL was then added to allow for storage and further chemical manipulations. As found for all commercially available nucleoside phosphoramidites, those prepared, herein, were a mixture of diastereoisomers at the phosphorus centre.

As expected from the increased stability of hydrolytically sensitive phosphorus reagents using ILs, following isolation, the phosphitylated nucleosides could be stored at room temperature, without the need for an inert gas atmosphere and showing no degradation after 5 days. This demonstrates the utilisation of ILs as stabilising/storage media for phosphitylated nucleoside derivatives and other reactive analogues currently under-utilised in oligonucleotide syntheses. Therefore, this approach not only allows facile access to diverse reactive building blocks, it also provides means to store them effectively.

Importantly, although ball milling has been used to enable solventless persilylation of nucleosides,[9,11] these reactions are much less air and moisture sensitive than those reported in this communication. Here, we describe the first combination of IL stabilisation with mechanochemical reaction conditions to simultaneously overcome problems of hydrolytic sensitivity *and* low reactant solubility. Specifically, we have combined these techniques to achieve enhanced reactivity, improved reaction profiles and increased yields in the phosphitylation of partially protected nucleosides with a range of hydrolytically labile chlorophosphoramidites. This represents a simple method to access, isolate and store reliably reagents known to be chemically labile, irrespective of the amine functionality. The method, therefore, will enable more efficacious RNA and DNA oligomerisation protocols to be developed.

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