Selective synthesis of chlorophosphoramidites using ionic liquids†

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Received 11th March 2009, Accepted 27th May 2009
First published as an Advance Article on the web 16th June 2009
DOI: 10.1039/b905000k

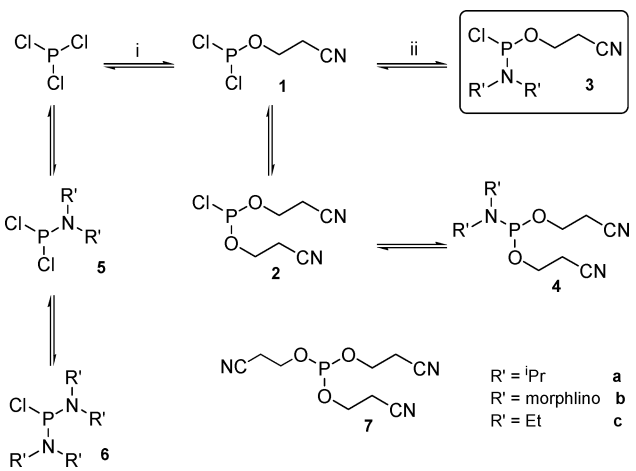
A range of chlorophosphoramidites have been prepared in ionic liquids and compared with material synthesised in molecular solvents. Through the use of ionic liquids as reaction media the moisture sensitivity and impurity issues hampering existing traditional synthetic routes have been eased. Not only can stock chemicals be used without purification, but the reactions may be conducted at room temperature and at high concentrations. Furthermore, reaction times are reduced and rapid addition of reagents is possible whilst retaining tight control over product selectivity. Beyond their role as reaction media, ionic liquids also present a unique storage medium for these highly moisture sensitive chlorophosphoramidites.

Introduction

Nucleoside chemistry represents an important area of research for drug discovery, with oligonucleotide-based therapeutics being evaluated in preclinical and clinical studies for the treatment of a variety of disease states such as cancer, cardiovascular disease, diabetes and inflammatory disease disorders.[1–3]

In order to prepare oligonucleotide-based therapeutics, nucleotides need to be synthesised by phosphorylation of partially protected nucleosides. Phosphorylation can be achieved in two ways; either by the introduction of a P^{V} reagent or by phosphitylation with a P^{III} reagent followed by oxidation.[4] Phosphitylation procedures with phosphoramidites and chlorophosphoramidites have been used most extensively owing to the increased reactivity compared with the P^{V} reagents and, with the development of phosphoramidite chemistry, oligonucleotide synthesis has been transformed into an efficient and automated process.[5,6] However, phosphoramidites and chlorophosphoramidites, in particular, are very sensitive to moisture and these compounds often require fresh preparation before use since they cannot be reliably stored over significant time periods. In general, the preparation of these derivatives requires the treatment of an excess of PCl_3 with a highly purified alcohol, with 3-hydroxypropionitrile being the most commonly used protecting group in oligonucleotide synthesis, producing an alkoxydichlorophosphine, which requires purification by distillation. Subsequent reaction with an appropriate amine, again requires purification by distillation before use. Although this route has been extensively optimised in molecular solvents, there is still significant room for improvement.[7] For example, 3-hydroxypropionitrile must be purified to remove the ethylene glycol contaminant *via* an expensive and complex synthetic purification sequence.

Ionic liquids are known to be viable alternatives to molecular solvents as they have negligible vapour pressure, unprecedented ability to dissolve a broad range of compounds both organic and inorganic in nature, and have a wide liquid range.[8] As such, ionic liquids are finding applications in synthesis owing to their utility in easing the preparation and recovery of targets in a number of cases.[9] In particular, bis{(trifluoromethyl)sulfonyl}imide ($[NTf_2]^-$) based ionic liquids represent a hydrophobic subsection of this class of solvents and, even when 'wet', by organic solvent standards, provide an environment which prevents hydrolysis of a range of hydrolytically unstable species, such as PCl_3 and $PN(R_1R_2)Cl_2$.[10,11] Herein, we report the facile preparation of a range of chlorophosphoramidite reagents using ionic liquids showing enhanced yields and selectivities over molecular solvents (Scheme 1).



Scheme 1 Chlorophosphoramidite preparation: i) Hünigs base (1 eq), 3-hydroxypropionitrile (1 eq), IL, 30 min. ii) Either Hünigs base (1 eq), R'_2NH (1 eq), 40 mins or R'_2NH (2 eq), 40 min.

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† Electronic supplementary information (ESI) available: Reaction mixture compositions for the systems illustrated in Figs. 1–3 and ^{31}P NMR chemical shifts. See DOI: 10.1039/b905000k

Results and discussion

Reaction of PCl_3 and 3-hydroxypropionitrile

In order to maximise atom efficiency with respect to both the conversion and yield of the product, stoichiometric ratios were maintained throughout the experiments with respect to PCl_3, alcohol, amine, and ionic liquid. The results in ionic liquid were compared with previously reported data for the preparation of chlorophosphoramidites in acetonitrile and diethyl ether.[12,13] $[NTf_2]^-$ based ionic liquids were employed as the reaction medium as they are known to provide a hydrolytically stable environment for the PCl_3 whilst maintaining a good balance between solubility and an inertness of the anion.[10] In many other ionic liquids, the anion is nucleophilic and has been found to react with the P^{III} reagents leading to side product formation thus limiting their use for this application. Tris(perfluoroethyl)trifluorophosphate ($[FAP]^-$) based ionic liquids provide an alternative which does not lead to nucleophilic displacement of chloride; however, PCl_3 has a low solubility in these ionic liquids again limiting their applicability. Therefore, in the majority of this work, only the effect of the cation was examined in detail.

The yields were determined from the ^{31}P NMR peak integration ratio, where the percentage yield corresponds to the ratio of the peak area of a given compound to the total of peak areas of all phosphorus containing species in a given NMR spectrum. This ratio corresponds to the molar speciation of the phosphorus containing compounds in that sample.

As previously reported, the reaction between PCl_3 and an amine generally gives quantitative yields of the desired PCl_2NR_2 product in short reaction times. The reaction of the aminochlorophosphine with an alcohol provides a possible route to the synthesis of chlorophosphoramidites. However, on reaction with the alcohol, significant hydrolysis and side product formation was observed. For example, the reaction of 3-hydroxypropionitrile with PCl_3 resulted in quantitative conversion of the aminochlorophosphine but only ~5% of the desired materials were formed. Therefore, in all subsequent reactions reported, herein, PCl_3 was first reacted with the alcohol followed by the amine.

The initial step in the chlorophosphoramidite synthesis was the reaction of PCl_3 and 3-hydroxypropionitrile in the presence of Hünigs base (Scheme 1). In molecular solvents, this reaction takes place at high dilution with external cooling of the reaction mixture. In addition to the extensive treatment of 3-hydroxypropionitrile, PCl_3 has to be distilled before use and used in a large excess to encourage the reaction to go to completion. The Hünigs base and the solvent of choice must also be dried or distilled before use. In contrast, the ionic liquid mediated reactions only require the ionic liquid to be dried under high vacuum for two hours before use. In the ionic liquid reactions the commercial reagents were used without pretreatment.

In the ionic liquids, PCl_3 was first solubilised with the subsequent addition of the Hünigs base. The mixture is then stirred vigorously for 5 min before the 3-hydroxypropionitrile is added. While the reaction is exothermic, no cooling of the system is required, and after 30 min an aliquot was removed for ^{31}P NMR analysis. Table 1 details the percentage yield distribution for all the ionic liquids used in this reaction. As can be seen from the data, in all cases the $P(OR)_2Cl$ derivative **2** was formed before all the PCl_3 had reacted. In addition, an *H*-phosphonate derivative was also formed during the alcohol addition. Interestingly, this formation was found to be reversible for some systems as shown by the absence of the ^{31}P NMR peaks corresponding to derivatives of these side-products in the final product composition after the subsequent amine addition.

Table 1 Relative product yields of **1** and **2** as a function of the ionic liquids determined by ^{31}P NMR integration and compared with the reaction performed in diethyl ether for the reaction of PCl_3 and 3-hydroxypropionitrile in the presence of 1 eq. Hünigs base at room temperature for 30 min

Ionic liquid[a] or molecular solvent	Composition (%)			
	PCl_3	$P(OR)Cl_2$ (**1**)	$P(OR)_2Cl$ (**2**)	*H*-phos
$[C_4mim][NTf_2]$	3	85	5	7
$[C_4dmim][NTf_2]$	4	89	7	
$[C_4mpyrr][NTf_2]$	3	83	8	6
$[C_4mpip][NTf_2]$	5	89	3	2
$[C_4py][NTf_2]$	3	90	5	2
Diethyl ether[b]		59		

[a] $[C_4mim]^+$ = 1-butyl-3-methylimidazolium, $[C_4dmim]^+$ = 1-butyl-2,3-dimethylimidazolium, $[C_4mpyrr]^+$ = 1-butyl-1-methylpyrrolidinium, $[C_4mpip]^+$ = 1-butyl-1-methylpiperidinium, $[C_4py]^+$ = 1-butylpyridinium, $[NTf_2]^-$ = bis{(trifluoromethyl)sulfonyl}imide. [b] Other products not reported and the reaction was performed at 0 °C with an 6 mol% excess of PCl_3.[13]

In comparison with commonly utilised methods for the preparation of **1**, the reactions in ionic liquids give excellent yields and with good selectivity. For example using $[C_4dmim][NTf_2]$ 89% of **1** was formed with no *H*-phosphonate contamination. Although it is possible to isolate **1** from the ionic liquid at this stage by distillation, addition of the nucleophilic amines was undertaken directly into the ionic liquid reaction mixture without workup to minimise the number of stages and to maximise yield. In contrast, the optimised reactions in molecular solvents, such as diethyl ether, require filtration of the amine hydrochloride salts that are formed during the reaction from the reaction mixture and isolation of the crude material, **1**, by evaporation of the solvent before further reaction with the amine. High reagent dilution is required in the molecular solvent to limit the effect of the salt waste on the conversion of PCl_3. Owing to the sensitive nature of **1**, careful work-up is also needed to reduce product hydrolysis.

The formation of chlorophosphoramidites in molecular solvents requires **1** to be dissolved in a large volume of anhydrous acetonitrile (or hexane) followed by the addition of freshly distilled nucleophilic amine in anhydrous acetonitrile (or hexane). In general, for most reactions diisopropylamine is used as the product formed displays a good balance between stability and reactivity. For example, using acetonitrile as the molecular solvent, the reaction of **1** with the nucleophilic amines is performed at −20 °C for 1.5 h and then at room temperature for a further 20 h resulting in the respective chlorophosphoramidites being formed in yields ranging from 50–85%.[12] Again the amine hydrochloride salt is filtered from the reaction mixture and the solvent concentrated to give the product **3**, with purification generally performed by distillation.

Careful storage of the material at low temperatures and under an argon atmosphere is required to prevent hydrolysis. For some materials, in particular the morpholino and diethylamino derivatives, storage is not a viable option and they must be used directly. This may be contrasted with the reactions performed in the ionic liquid wherein amine (1 eq) and Hünigs base (1 eq) or amine (2 eq) was added to the ionic liquid reaction mixture without any workup following the formation of **1**. It should be noted that the amine hydrochloride salt is soluble in the ionic liquid allowing high concentrations of reagents to be used. In contrast, in the molecular solvents the slurry formed limits the concentration able to be utilised due to mass transfer limitations which reduces the reaction rate. Figs. 1–3 show the percentage yield distribution for the reaction mixtures as determined by ^{31}P NMR analysis of samples taken 40 min after amine addition to the ionic liquid reaction mixture without any workup following the formation of **1** for diisopropylamine, morpholine and diethylamine, respectively.†

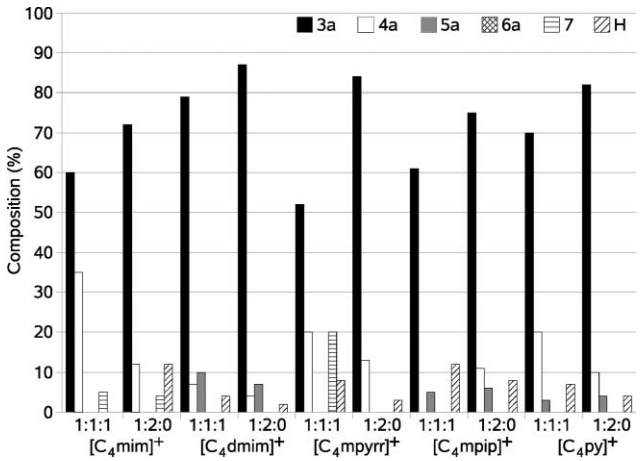


Fig. 1 Percentage yield for the reaction 1 with diispropylamine in $[NTf_2]^-$ based ionic liquids as a function of the cation with either a reaction ratio of 1:1:1 or 1:2:0 PCl_3:Amine:Hünigs Base. H corresponds to hydrolysis.

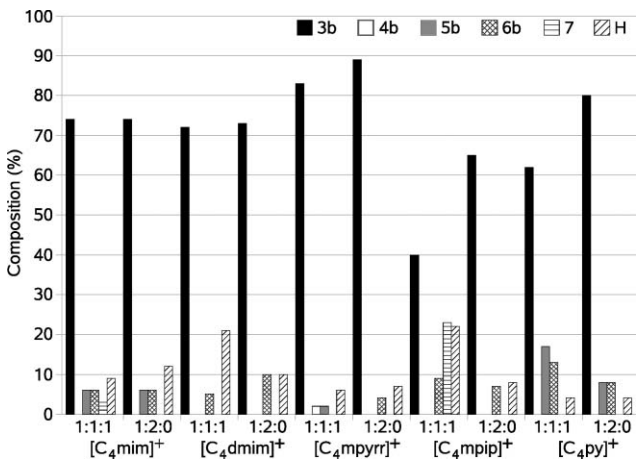


Fig. 2 Percentage yield for the reaction of 1 with morpholine in $[NTf_2]^-$ based ionic liquids as a function of the cation with either a reaction ratio of 1:1:1 or 1:2:0 PCl_3:Amine:Hünigs Base. H corresponds to hydrolysis.

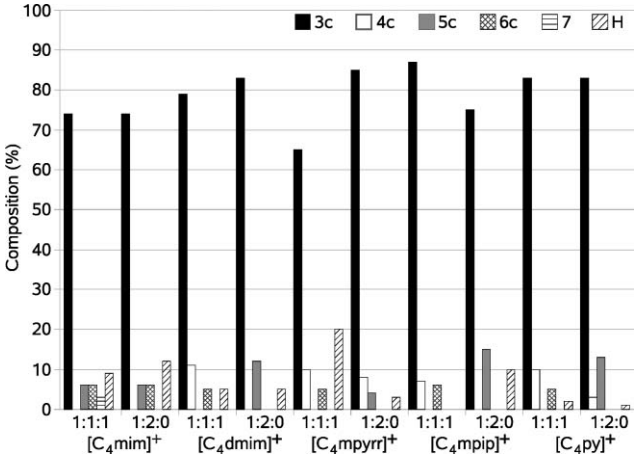


Fig. 3 Percentage yield for the reation of 1 with diethylamine in $[NTf_2]^-$ based ionic liquids as a function of the cation with either a reaction ratio of 1:1:1 or 1:2:0 PCl_3:Amine:Hünigs Base. H corresponds to hydrolysis.

The factors determining the selectivities observed are a complex combination of the nucleophilicity of the amine and the alcohol as well as the pK_a of the amine and the base in the ionic liquid solvent and these determine the equilibrium constants for the reaction matrix shown in Scheme 1. The underlying reason for the enhanced chemoselectivity in the ionic liquids compared with the molecular solvents is not clear. It is possible that the ionic liquid provides a medium whereby the nucleophilic molecules and the PCl_3 become well dispersed from other like molecules, as found in the case of the water, and, therefore, this limits the chance of multiple nucleophilic attack on the PCl_3 by amine, for example. This is aided by the higher viscosity of the ionic liquid which reduces the diffusion coefficient and slows intermixing of reagents. Although the mechanism is not well understood to date, it is clear that the predominant product formed in all cases is the desired $P(OR)(NR'_2)Cl$ material irrespective of the ionic liquid used, the nature of the amine and whether or not Hünigs base is present. However, the order of addition of reagents was found to be critical in optimising the formation of **3**.

In general, reactions using 2 equivalents of amine give higher yields of the desired products **3a-c** than the corresponding reaction involving 1 equivalent of both Hünigs base and amine. Interestingly, in some cases the dialkoxy product ($P(OR)_2(NR'_2)$, **4a-c**) is formed but in a much higher yield than would be expected based on the product from the first reaction step (Table 1) indicating that the entire system is under equilibrium. For the diethylamine set of products, the 1:1:0 conditions generally formed $P(NR'_2)_2Cl$ derivatives in the product mixture, whereas the 1:2:0 reagent ratio lead to $P(NR'_2)Cl_2$ type species. This trend was not observed for the other amine systems with the $P(NR'_2)_2Cl$ derivative not being observed for diisopropylamine. This difference may be associated with the increased steric hindrance of the amine used. In addition, $P(OR)_2(NR'_2)$ type species was not observed using morpholine, and the formation of side products for this amine show no significant trend between reaction conditions or ionic liquid employed.

Of the three products formed, diisopropylamino derivative is commercially available, but the quality of the product can vary.

The other two derivatives are too unstable to store, and as such are not available to purchase.

Isolation and characterisation

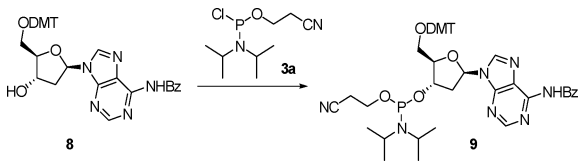
Two main methods were employed for the isolation of **3** from the ionic liquid mixture, namely extraction and distillation. Solvent extraction proved to be a useful method of isolating **3a** from the reaction mixture. Using diethyl ether the product could be isolated; however, some hydrolysis did occur on removal from the ionic liquid. Unfortunately it was not possible to isolate the derivatives **3b** and **3c** using this methodology. Both compounds are highly sensitive to hydrolysis, and once removed from the ionic liquid, significant hydrolysis occurred and little product remained intact.

Distillation proved to be a more useful method for the isolation of these sensitive materials with both the diisopropylamino and diethylamino compounds successfully removed from the reaction mixture cleanly without decomposition of the ionic liquid. The diisopropyl derivative **3a** was isolated; however, as with solvent extraction, the diethyl derivative **3c** decomposed immediately due to its high sensitivity. This could be overcome by distillation of the product material directly into the reaction flask, or into fresh ionic liquid for storage purposes until required. Using distillation, it was also possible to distil the chlorophosphoramidites into alternative ionic liquids, namely the tris(perfluoroethyl)trifluorophosphate ([FAP]$^-$) based ionic liquids. Ionic liquids based on this anion are even more hydrophobic than those based on the [NTf_2]$^-$ anion and this media provided a more stable environment for these sensitive reagents. As described above, due to the insolubility of PCl_3 in [FAP]$^-$ based ionic liquids, it was not possible to undertake the full synthetic procedure in this media. Due to the high thermal instability of the morpholino derivative, **3b**, distillation was not possible. However, preliminary investigations into the use of supercritical CO_2 extraction have shown promise for this and the other systems and are currently being optimised. In addition, the use of thin film distillation could also improve upon the isolated yields of the distillable materials.

Stability and phosphitylation

All reaction mixtures were monitored over an extended period of time to establish the stability of the chlorophosphoramidites, not only when distilled into fresh ionic liquids, but also within the reaction mixtures. In agreement with the previous findings for aminochlorophosphines,[11] the chlorophosphoramidites once formed were found to be stable with respect to hydrolysis within the ionic liquid, illustrating that this class of solvent not only represents an excellent medium for the formation of these sensitive reagents but also offers exceptional storage qualities. Furthermore, no decomposition of the chlorophosphoramidite reagents was observed after being stored in the ionic liquid at room temperature for several weeks even in the absence of an inert atmosphere.

Further studies showed that the chlorophosphoramidite, **3a**, dissolved in [C_6mim][FAP] could be added to anhydrous acetonitrile and ^{31}P NMR indicated no degradation of material over 72 h in acetonitrile at room temperature in the absence of an inert atmosphere. Using the stability provided by the ionic liquid, a simple phosphitylation reaction was undertaken with the phosphoramidite and a protected (Scheme 2).



Scheme 2 Phosphitylation reaction of protected 2-deoxyadenosine in DCM with **3a** in [C_6mim][FAP] in the presence of Hünigs base.

Before use the nucleoside was azeotroped and dried under high vacuum to reduce the potential for the co-crystallised water to hinder the reaction; however, the Hünigs base was not distilled before use. The reaction was performed in anhydrous dichloromethane, as reactions in anhydrous acetonitrile only resulted in deprotection of the trityl protecting group. After 1.5 h at room temperature, quantitative conversion of the chlorophosphoramidite to a diastereotopic mixture of phosphitylated nucleosides with chemical shifts at 149 and 150 ppm by ^{31}P NMR was found. After purification by column chromatography the phosphitylated nucleotide was isolated in 92% yield, illustrating that the ionic liquid stabilised chlorophosphoramidite could be successfully employed for the synthesis of nucleotides.

Conclusions

[NTf_2]$^-$ based ionic liquids provide a media in which chlorophosphoramidites can be prepared without extensive purification of the starting materials and external cooling of the reaction mixture. The products were formed with high selectivity without significant hydrolysis of the product. These selectivities are difficult to achieve in molecular solvents and normally require the use of excess reagents, derivatisation of the starting materials and careful control of the reaction temperature and concentration. The ionic liquid reactions were also carried out at high concentration, reducing the amount of solvent required when compared with the analogous reaction in molecular solvent.

The chlorophosphoramidites were found to be readily distilled from the reaction mixture as isolated compounds or into a stabilising clean ionic liquid. In particular, distillation into [FAP]$^-$ based ionic liquids provided a medium in which the reagents could be added directly to molecular solvents, without isolation, providing a stable environment to protect these sensitive materials before use. This has been utilised to prepare phosphitylated nucleosides in quantitative yield without isolation of the intermediate molecules.

Nucleoside chemistry is an extremely important area of research, but one in which the solvent choice is severely compromised by poor solubility of the reagents. The solubility of nucleoside and nucleic bases in ionic liquids have already been reported,[14–16] Using the methodology developed herein, there is scope for the complete preparation of a wide range nucleotides in ionic liquids to be realised with high selectivity.

Experimental

General procedures

The product distribution of the reactions of PCl_3 for the synthesis of chlorophosphoramidites was examined *in situ* by ^{31}P NMR and ^{1}H-^{31}P coupled NMR. Large scale reactions were carried out in order to establish a protocol for product isolation and to examine the product stability in ionic liquids. Five sets of parallel experiments were performed using 1-butyl-3-methylimidazolium bis{(trifluoromethyl)sulfonyl}imide ([C_4mim][NTf_2]), 1-butyl-2,3-dimethylimidazolium bis{(trifluoromethyl)sulfonyl}imide ([C_4dmim][NTf_2]), 1-butyl-3-methylpyrrolidinium bis{(trifluoromethyl)sulfonyl}imide ([C_4mpyrr][NTf_2]), 1-butyl-3-methylpiperidinium bis{(trifluoromethyl)sulfonyl}imide ([C_4mpip][NTf_2]), and 1-butyl-pyridinium bis{(trifluoromethyl)sulfonyl}imide ([C_4py][NTf_2]). The ionic liquids used were prepared in house using standard literature methods[17] from the appropiate halide salt. 1-hexyl-1-methyl-imidazolium tris(pentafluoroethyl)trifluorophosphate ([C_6mim][FAP]) was supplied by Merck KGaA. All ionic liquids were dried under high vacuum for 2 h prior to use. The water content and bromide content were measured for each ionic liquid using Karl Fischer titration and ion chromatography, respectively. In each case the bromide levels were below 5 ppm and the water content for the dried ionic liquids were < 0.04 wt%.

For each reaction, the mole ratio of PCl_3 (Aldrich, 98%), the nucleophilic amine and the base, diisopropylethylamine (Hünigs base) (Aldrich, 99%) were varied. The mole ratio of PCl_3 and 3-hydroxypropionitrile (Aldrich, 99%) was kept constant. The amines investigated were diisopropylamine, morpholine and diethylamine and were obtained from Aldrich. Diisopropylamine was distilled over calcium hydride prior to use. PCl_3, 3-hydroxypropionitrile, Hünigs base and the remaining amines were used as supplied.

Spectroscopic details

All the ^{31}P, ^{1}H-^{31}P nuclear magnetic resonance spectra were recorded on a Bruker Bruker Avance 300 or 500 at 25 °C. For ionic liquid samples an aliquot was transferred directly into the NMR tube with no addition of deuterated solvents. The ^{31}P-NMR chemical shifts were recorded in parts per million (ppm) relative to an external probe (sealed capillary inside the NMR tube sample) of triethylphosphonate ($PO(OEt)_3$) in $CDCl_3$ (solvent used for locking/shimming optimisation). The $PO(OEt)_3$ probe was referenced to 0.2 ppm. For the nucleotide the NMR was recorded in $CDCl_3$ referenced to 0.00 ppm using TMS for the ^{1}H NMR and 77.00 ppm using $CDCl_3$ for the ^{13}C NMR.

General experimental conditions

To a stirred solution of PCl_3 (1 eq) in dried ionic liquid (1 eq) under an atmosphere of argon was added Hünigs base (1 eq). The solution was stirred vigorously for 5 min and 3-hydroxypropionitrile (1 eq) was added. The reaction mixture was stirred for 30 min then either nucleophilic amine (2 eq) was added or Hünigs base (1 eq) and nucleophilic amine (1 eq). Reactions were generally complete after a further 40 min.

(2-Cyanoethoxy)-*N*,*N*-diisopropylamino-chlorophosphoramidite 3a. Distilled from [C_4dmim][NTf_2] at 88–90 °C at 0.01 mmHg, or extracted with diethyl ether to give a colourless liquid (46%). ^{1}H NMR (300 MHz, $CDCl_3$) 1.26 (12H, d, J 6.8 Hz, 4 CH_3), 2.73 (2H, t, J 6.25 Hz, OCH_2CH_2CN), 3.68–3.86 (2H, m, 2 CH), 4.05 (2H, dt, J 8.17, 6.25 Hz, OCH_2CH_2CN). ^{13}C NMR (75 MHz, $CDCl_3$) 19.7 (CH_3), 46.5 (d, CH), 47.8 (CH_2), 60.8 (d, CH_2), 117.3 (CN). ^{31}P NMR (121 MHz, $CDCl_3$) 181 (P(OR)(N^iPr_2)Cl).

(2-Cyanoethoxy)-*N*-morpholino-chlorophosphoramidite 3b. It was not possible to distil the morpholino derivative due to thermal instability or extract with molecular solvent due high hydrolytic instability. Selected data from crude reaction mixture: ^{13}C NMR (75 MHz, $CDCl_3$) 16.7 (CH_2), 58.6 (d, CH_2), 66.1 ($N(CH)_2$), 71.2 (CH_2), 118.1 (CN). ^{31}P NMR (121 MHz, $CDCl_3$) 168.6 (P(OR)(N^iPr_2)Cl).

(2-Cyanoethoxy)-*N*,*N*-diethylamino-chlorophosphoramidite 3c. Distilled from [C_4dmim][NTf_2] at 90–92 °C at 0.01 mmHg, into fresh [C_4dmim][NTf_2] (44%). Data from mixture of ionic liquid and product. ^{1}H NMR (300 MHz, $CDCl_3$) 1.12 (6H, t, J 9.0 Hz, 2 CH_3), 2.72 (2H, t, J 6.0 Hz, OCH_2CH_2CN), 3.09–3.16 (4H, m, 2 NCH_2), 4.03 (2H, dt, J 8.0, 6.0 Hz, OCH_2CH_2CN). ^{13}C NMR (75 MHz, $CDCl_3$) 18.2 (CH_3), 39.8 (d, J 18.5 Hz, CH_2), 42.5 (CH_2), 61.5 (d, J 17.5 Hz, NCH_2), 118.3 (CN). ^{31}P NMR (121 MHz, $CDCl_3$) 176.9 (P(OR)(NEt_2)Cl).

General procedure for nucleoside phosphitylation[18]

Protected 2-deoxyadenosine, **8**, (1 eq) was azeotroped with toluene (3 times) and dried under high vacuum for 3 h before use. The nucleoside was taken up in anhydrous DCM and 2 eq of Hünigs base was added. The solution was stirred for 10 min then chlorophosphoramidite, **3a**, in [C_6mim][FAP] (2.5 eq) was added to the reaction mixture. After 45 min, the ^{31}P NMR showed peaks at 149 and 150 ppm. After a further 1 h the mixture was concentrated *in vacuo* and purified by column chromatography (1:1–0:1 hexane:ethyl acetate, 1% NEt_3) to yield the protected nucleoside, **8**, as a white solid (92%, 2:1 diastereotopic mixture).

***N6*-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine-3′-*O*-[*O*-(2-cyanoethyl)-*N*,*N*′-diisopropylphosphoramidite 9.** ^{1}H NMR (500 MHz, $CDCl_3$) 1.12–1.22 (12H, m, 4 CH_3), 2.48 (2H, t, J 6.4 Hz, OCH_2CH_2CN-a), 2.63 (2H, t, J 6.3 Hz, OCH_2CH_2CN-b), 2.75–2.78 (1H, m, H-2′), 3.31–3.3.63 (7H, m, H-5′, NC*H*, OCH_2CH_2CN), 3.76 (3H, s, OCH_3-a), 3.78 (3H, s, OCH_3-b), 4.28–4.35 (1H, m, H-4′), 4.76–4.82 (1H, m, H-3′), 6.49–6.55 (1H, m, H-1′), 6.76–6.82 (4H, m, ArCH), 7.17–7.30 (6H, m, ArCH), 7.38–7.7.41 (2H, m, ArCH), 7.51–7.62 (4H, m, ArCH), 8.02 (2H, d, J 12.0 Hz, ArCH), 8.20 (1H, s, H-2-a), 8.22 (1H, s, H-2-b), 8.75 (1H, s, H-8-a), 8.76 (1H, s, H-8-b), 8.97 (1H, br s, NH). ^{13}C NMR (125 MHz, $CDCl_3$) 22.9–23.1 (CH_3), 24.66–24.68 (CH_2), 39.6 (NCH), 43.3–43.3 (C-2′), 50.37 (CH_2), 55.28 (OCH_3), 58.1 (C-4′), 63.4 (C-5′), 77.2 (C-3′), 84.7 (C-1′), 86.5 (C), 113.1 (ArCH), 117.5 (CN), 122.0–132.8 (ArCH), 135.6, 135.7 (ArC), 141.8 (C-8), 144.4, 149.4 (ArC), 152.6 (C-2), 158.5 (C=O). ^{31}P NMR (121 MHz, $CDCl_3$) 149.9, 150.0.

HRMS (ES, M + H^+) calculated for $C_{47}H_{53}N_7O_7P$ 858.3744, found 858.3756.

Acknowledgements

We would like that thank QUILL for funding, Merck KGaA for financial support and the donation of ionic liquids and the EPSRC for funding under a Portfolio Partnership.

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