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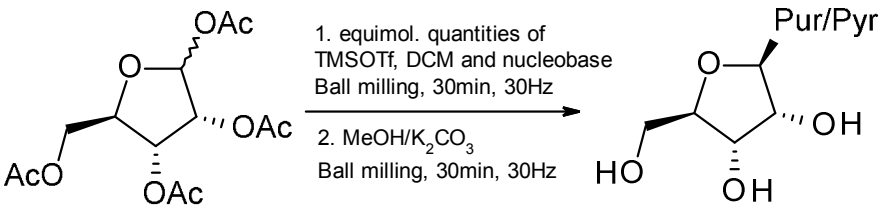

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A range of nucleosides have been synthesised utilising a solventless approach to Vorbrüggen glycosylations aided by mechanochemistry.

Nucleosides and nucleotides are essential in a myriad of cellular signalling and metabolic pathways.[1] Consequently, these naturally occurring building blocks and their analogues have been the target of chemical synthesis for many years in order to probe their applicability as therapeutic agents for a variety of disease states, including a variety of cancers, viral infections and inflammatory disorders.[2] The glycosylation reaction between nucleobases and activated sugars to access β-ribonucleosides have remained largely unchanged since its introduction more than 90 years ago. In particular, the silyl Hilbert- Johnson reaction, also known as the Vorbrüggen reaction, which employs the use of Lewis-acid catalysts, has been extensively explored with regards to this transformation.[3]

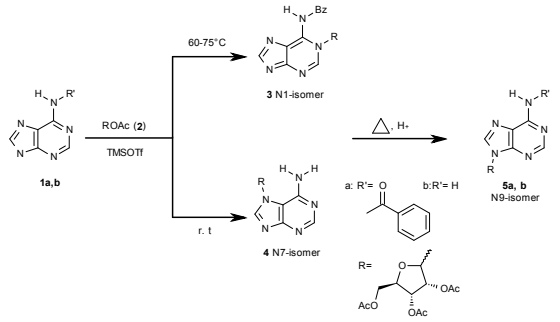

Figure 1 Ribosylation of N^6-benzoyladenine **1a** and adenine **1b** resulting in different kinetic product distributions, depending on reaction conditions, and which equilibrate under thermodynamic promoted conditions.[4]

A typical nucleoside Vorbrüggen-type synthesis involves a three-step process: the silylation of the nucleobase; the Lewis acid catalysed condensation reaction between the silylated nucleobase and the adequately protected sugar; and finally the removal of the remaining protecting groups to yield the nucleoside. Whilst popular, Vorbrüggen reaction conditions applied to purine nucleobases require prolonged heating to obtain the thermodynamically favoured N9 isomers, as the ribosylation occurs *via* different kinetic reaction intermediates depending on the reaction temperature, the nature of the sugar (1-halo *vs.* 1-*O*-acetyl) and the nature of N6-protecting groups, Figure 1.[4,5] Consequently, there is a need for a more versatile protocol towards the ribosylation of heteroaromatic bases, and whereby the use of acetonitrile or dichloroethane as solvent can be minimised. Mechanochemistry has been an integral part of material science since the beginning of modern chemistry. More recently, mechanical milling has been fruitfully utilized in organic synthesis under solvent free- conditions where the need for hazardous organic solvents has been circumvented and waste generation has been minimised.[6] Such examples include the protection and functionalisation of sugars,[7] the protection and phosphitylation of nucleosides,[8] the preparation of peptide linkages,[9] as well as the rapid and economic preparation of pyrophosphate linkages.[10] Despite this recent success in the derivatisation of biologically relevant molecules, the synthesis of nucleosides themselves *via* mechanical milling remains relatively unexplored. Herein, we report the solvent free synthesis of nucleosides using ball milling conditions. Regioselective ribosylation was rapid (30 min) and achieved using stoichiometric amounts of nucleobases, 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**2**) and trimethylsilyl triflate (TMSOTf). Moreover we have demonstrated that prior silylation of the nucleobase was not required in some cases under ball milling conditions, thus mitigating the need for the first key synthetic step of the Vorbrüggen glycosylations.

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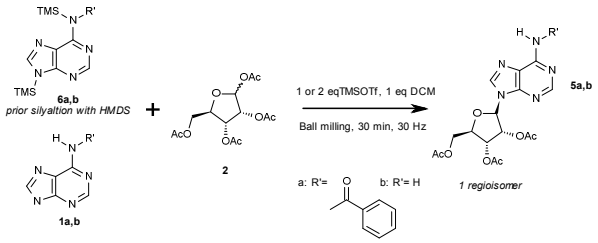

Figure 2 Schematic showing the glycosylation reaction between prior silylated adenine derivates **6a,b** and adenine derivatives **1a,b** with **2** and TMSOTf using mechanochemistry. ‡

The initial Vorbrüggen glycosylations using ball milling were attempted using the pre-silylated bases, **6a,b**, as prior silylation is a prerequisite for the analogous solvent based protocol. This is thought to enhance the lipophilicity and nucleophilicity of the nucleobases, which often exhibit poor solubility in most conventional solvents.[11] The silylation was conducted using hexamethyldisilazane (HMDS) according to standard literature procedures.[12] Satisfyingly, for the reaction of **6a** with the tetraacetate sugar **2** in the presence of TMSOTf using the mechanochemical conditions outlined in Figure 2, N^6-benzoyl adenosine **5a** was obtained quantitatively as the sole regioisomer as confirmed by ^{1}H NMR in 30mins. It is well reported in the literature that acylation of the N^6-position encumbers glycosylation at N7 with enhanced N9 regioselectivity.[5] In order to establish if N^6-acylation was necessary under our ball milling procedure, adenine **6b** was ball milled in equimolar ratios with **2** and TMSOTf. Once again, only one regioisomer, adenosine **5b**, was obtained. In contrast, under the analogous solvent based conditions reported in the literature, a mixture of **3** (figure 1) and **5a** have been reported for the reaction between **6a**, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl β-L-ribofuranose and TMSOTf.[13] In an attempt to mitigate the need for prior silylation of the nucleobases, we explored the possibility of the silylation of **1a** and **1b** *in-situ* using two equivalents of TMSOTf, Figure 2. Grinding commercially available nucleobases **1a** and **1b** in the ball mill for 30 min with 2 equivalents of TMSOTf and 1 equivalent of **2**, afforded ribonucleosides **5a** and **5b** quantitatively (Table 1, entries 1 and 2). Two equivalents of TMSOTf was found to be optimal for reactions where there was no prior silylation of the nucleobases. For instance, using **1b** as an exemplar, when one equivalent of TMSOTf was used for 30 min at 30 Hz, a low isolated yield (18 %) was observed, with no unreacted sugar **2** recovered following work-up. This is attributed to the reactivity of **2** towards TMSOTf under milling conditions to generate the oxycarbanion, which then degrades in the absence of the more reactive silylated form of nucleobase **1b**. It can therefore be concluded that when in competition, the generation of the oxycarbocation by TMSOTf precedes the nucleobase silylation under milling conditions. As such, two equivalents of TMSOTf are required to enhance the lipophilicity and nucleophilicity of the nucleobases and for activation of the sugar **2**, resulting in the quantitative conversion of the sugar into the corresponding nucleosides (Table 1, entries 1-9). Of note, under these milling conditions, the biologically relevant isomer, the N9 isomers **6a** and **6b** (figure 1) formed rapidly and selectively, in contrast to the solution reaction conditions. James *et al* have reported that the temperature in the grinding chamber increases with increasing grinding frequencies, and as a consequence of frictional heating. They proposed that an increase in grinding frequency increased the reaction rates due to the increased frequency of reactive contacts between reactants. They proposed that milling could induce the reaction medium to adopt a fluid-like character due to the mobility of the particles, thus it could be thought of as a pseudo fluid.[15] In the case of the present glycosylation reactions, milling was performed at 30 Hz. Therefore an increase in temperature over the course of these reactions can be anticipated; promoting early thermodynamic control. Importantly, for all milling reactions, one molar equivalent of dichloromethane had to be used to ensure a paste-like reaction medium and achieve an efficient mixing of reagents, as in the absence of this co-reagent, the reaction was severely compromised. The liquid-assisted grinding (LAG) significantly improved the glycosylation reaction rates by enhancing the effective mixing and reactive collisions of individual molecules.[14] Combined, these two observations help rationalise the rapid formation of N^6-benzoyl adenosine **5a** and adenosine **5b** as the sole reaction products.

Table 1 Vorbrüggen reaction between **2** and nucleobases using ball milling

Entry	Nucleobase	Prior silylation[a] 1 eq. TMSOTf	Isolated Yield (%)[b]	No prior silylation 2 eq. TMSOTf	Isolated Yield (%)[b]	Conversion (by ^{1}H NMR)
1	N^6-benzoyladenine **1a**	N9 isomer only	87	N9 isomer only	82	quant.
2	adenine **1b**	N9 isomer only	85	N9 isomer only	80	quant.
3	N^2-isobutyrylguanine **7**	multiple isomers	-	multiple isomers	-	quant.
4	Cytosine **8**	SM recovered	-	SM recovered	-	-
5	N^4-benzoylcytosine **9**	N1 isomer only	81	N1 isomer only	79	quant.
6	Thymine **10**	N1 isomer only	89	N1 isomer only	83	quant.
7	Uracil **11**	N1 isomer only	84	N1 isomer only	80	quant.
8	hypoxanthine **12**	Multiple isomers	-	multiple isomers	-	quant.
9	6-chloropurine **13**	N9 isomer only	78	multiple isomers	-	quant.

[a]Prior silylation conditions: HMDS, cat. $(NH_4)_2SO_4$, 140°C, overnight;[b] Products were isolated *via* an aqueous work-up and extraction with DCM, drying with $MgSO_4$ and concentration under high vacuum

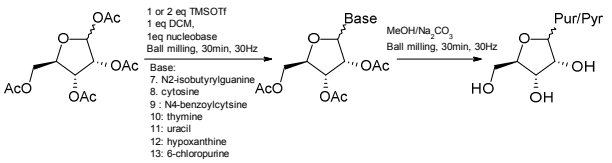

Figure 3 Mechanochemical synthesis of purine and pyrimidine nucleosides.

The mechanochemical approach described in Figure 2 was readily extended to include the reaction of **2** with various nucleobases, Figure 3. ‡ As such, other nucleobases in the purine series, namely **7**, **12** and **13**, were subjected to ball milling with **2** in the presence of TMSOTf. For all reactions performed, the requirement for prior silylation, in addition to *N*- protection was investigated. In general, the reactions proceeded quantitatively to give a single regioisomer as described in Table 1, entries 3-9. The reaction of prior silylated 6-chloropurine **13** with **2** and 1 equivalent of TMSOTf afforded the corresponding nucleoside **14g** in 78 % isolated yield, Table 1, entry 9. This is directly comparable with the 77 % yield previously reported by Potter *et al* for the preparation of 6-chloropurine riboside.[17] Unfortunately, under milling conditions multiple isomers were observed by ^{1}H NMR for the reaction of N^2-isobutyrylguanine **7** and hypoxanthine **12**. This outcome was attributed to the reduced nucleophilicity of the purines **7** and **12**, which often leads to a mixture of regioisomers.[18] Satisfyingly, the newly developed ball milling procedure was particularly amenable to pyrimidine based nucleobases, with the exception of unprotected cytosine **8**, Table 1, entries 4-7. For the pyrimidine nucleobases **9-11**, nucleosides **14 c-e** were isolated in high yields (79-83 %) when a one pot, two step approach was used *via 'in-situ'* silylation of the nucleobases using 2 equivalents of TMSOTf. The yields achieved herein are comparable to the literature procedures for pyrimidine nucleoside synthesis.[3, 19] These mechanochemical reaction conditions are remarkably faster than the analogous solvent-based protocols, and the yields are equal or superior to that reported by Bookser *et al* who developed a microwave accelerated glycosylation process.[16] Although the microwave-enabled synthesis was rapid (5 min), the procedure required high temperatures (130°C), prior silylation and a molar excess of both TMSOTf and acetonitrile. In contrast, the present ball milling protocol does not require heating, enables '*in-situ*' silylation of the nucleobases and allows for stoichiometric ratios of solvent and reagents to be utilised and while conducted on 10-20mmole scale, can be easily scaled up. Finally, as an exemplar deacetylation of **5b** was also conducted under milling conditions, whereby potassium carbonate in methanol (10%mass/vol; 5-10 eq. MeOH) were added to the crude nucleoside and the resulting mixture was milled for 30 mins. This rapid procedure also facilitated the isolated nucleoside in the absence of acetamide from standard ammonia deprotection conditions.

In conclusion, the continued demand for nucleoside-based therapeutics and research tools means that improved and cost effective approached are still in demand. By utilising ball milling technology, we have developed a rapid and efficient protocol for the preparation of purine and pyrimidine nucleosides. This method provides significant potentials for further applications to the preparation of diverse libraries of nucleoside analogues.

Notes and references

‡ Typical protected nucleoside ball milling procedure: a 25 cm^3 PTFE ballmilling vessel was charged with tetraacetate sugar **2** (1eq, typically 10mmol), TMSOTf (2eq), nucleobase/prior silylated nucleobase (1 eq), DCM (1 eq) and a PTFE ball bearing. The vessel was shaken with a Retsch MM 200 mixer mill for 30 min at 30 Hz. The crude material was washed from the reactor vessel with a small amount of DCM, washed with water, dried over $MgSO_4$ and concentrated. For the *in situ* deacylation step, methanolic potassium carbonate (5-10 mol. eq) was added to the crude reaction mixture and the vessel was milled for a further 30min. The crude material was washed from the reactor vessel with a small amount of water and residual organic materials were removed by extraction with ethyl acetate. The aqueous fraction was freeze-dried to yield the fully deprotected nucleosides.

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