Facilitation of nucleoside and nucleotide chemistry by ball milling


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Nucleoside “click” reactions under solvothermal conditions or by ball milling in a copper vessel

Copper (I)-catalysed azide-alkyne cycloaddition (CuAAC) reactions have found wide utility in nucleoside and nucleotide derivatisation. Typically, reactive Cu(I) is either generated in situ from a Cu(II) source or added directly in the presence of a stabilising ligand although in their original report, Sharpless and coworkers also described the capacity of cycled copper metal turnings to promote regioselective 1,3-dithiane formation. Based upon these observations, we have investigated “click” cycloaddition reactions between 5′-azido-5′-deoxythymidine (4) and mono- (5a-c) or bis- (5d) N-propargylamidine-substituted azobenzenes under different reaction conditions (SCHEME 2). In solution, high to quantitative yields were achieved using 5 mol% Cu(I) in the presence of TBTA; in high speed vibration ball milling (H&VBM) using a 3.32 mm diameter copper ball (62 mg) overnight in the presence of ethyl acetate lead to complete consumption of the 5′-azido nucleoside with clean conversion to the corresponding 1,3-dithiane without contamination by copper (TABLE: Method B). In contrast, more rapid reaction was induced in a more capacious copper vessel using higher energy impacts but this was at the cost of the physical integrity of the metal surface (FIGURE 1b).

Summary
In this study we have compared the efficiency of CuAAC reactions using ball milling and in solution and, in the former case, the click reaction can be taken to completion in the absence of cupric ion contamination of the products following prolonged vibration in a copper vial at high frequencies.

Preparation of a novel pyrophosphorothiolate-linked dinucleoside (AppsA) in a ball mill
Nucleoside-phosphorothiolate monomers (RSP-OT) are susceptible to hydrolytic P-S bond cleavage especially under acidic conditions and therefore are incompatible with the prolonged reaction times typically required for solution-phase pyrophosphate coupling under Khorana conditions. CDI-mediated coupling is more rapid and was used by Patel and Eckstein to activate 5′-thioadenosine-5′-phosphorothioate monomeric in order to effect coupling with [P(OH)2(Bu)NH2] but the corresponding triphosphate was isolated in low yield. 5′-Thioadenosine 5′-pyrophosphate (P–5′)-adenosine (AppsA - 11) was prepared over a series of steps (SCHEME 3). Attempted nucleophilic displacement of chloride from 5′-Cl (7) using sulfur nucleophiles in a steel vessel (FIGURE 1d) resulted in its corrosion and product contamination as reported by other workers. In contrast, reaction of p-anisyl-mercaptan in zirconia-lined vessels (FIGURE 1c) gave the thioether (8) cleanly. One pot desilylation and disulfide exchange enabled isolation of the activated disulfide (9) in good yield. For the key pyrophosphate coupling, initial Michaels-Arthozen coupling of 9 was performed in the presence of excess silylating agent. Under such conditions, the putative intermediate (10) is relatively stable (less than 5% degradation was observed by 19P-NMR over 6 days at room temperature). By transferring the reaction mixture to the vessel, evaporating and adding sufficient water in addition to other reagents (step IV), desilylation and pyrophosphate coupling was effected in one pot using vibration ball milling (FIGURE 2). Pure AppsA (11) was isolated following RP-HPLC purification in 40% yield (from 9) and characterised by NMR (FIGURES 3 and 4).

Facilitatration of nucleoside and nucleotide chemistry by ball milling

Olga Eguaojie,1* Andrew J. Cummings,1 Francesco Ravalico,1 Kegan I. S. McColgan-Bannoun,1 Matthew R. Shannon,1 Patricia M. L. Martin,1 Christopher J. Law,1 and Joseph S. Vyle1

1School of Chemistry and Chemical Engineering, Queen’s University Belfast, David Keir Building, Stranmillis Road, Belfast BT9 5AG, UK.
2School of Biological Sciences, Queen’s University Belfast, Medical Biology Centre, Lisburn Road, Belfast BT9 7BL, UK.

ceguaiojie@qub.ac.uk

Introduction

• Nucleic acid chemistry is a particularly attractive target for developing alternatives to molecular organic solvents as reaction media. Typically, the amphiphilic nature of these compounds requires the use of solvents such as THF, DMF or pyridine. Such solvents are carcinogenic and/or highly toxic, require energy-intensive processes to remove and typically have been dried over calcium hydride or phosphorus pentoxide and distilled prior to use in reactions including moisture-sensitive reagents.
• Due to the minimal solvent requirements involved, ball milling provides a potential alternative to solvothermic nucleoside chemistry as recently demonstrated by rapid, atom-economic pyrophosphate coupling (SCHEME 1). In the absence of solubility issues, phosphite acceptors could be used as their salts (2a-f). Moderate to high yields were achieved within hours using a vibration ball mill (FIGURE 1a).
• In the following ball milling studies we examined:
  1) “click” cycloaddition reactions between nucleoside 5′-azides and mono- or bis-alkynes in a copper vessel (FIGURE 1B) or using established solvothermal reaction conditions.
  2) pyrophosphate coupling with a 5′-adenosine-5′-monophosphorothiolate (AMP-M) with 5′-diotheno monophosphoromorpholidate (AMP-β) resulted in its corrosion and product contamination as reported by other workers.

Summary
In this study we have demonstrated the capacity of ball-milling to facilitate nucleoside substitution upon a nucleoside in the absence of DMF and synthesised a novel pyrophosphorothiolate-linked dinucleoside (AppsA) via an acid-sensitive phosphorothiolate monomer using ball milling to effect the key one pot hydrolytic desilylation/ pyrophosphate coupling. As 5′-linked structures are substrates for ligases, this may provide a more efficient (biochemical) route for 5′-phosphorothiolate linkages than that via the monomer.6,8

Table: Product Yield / % Cu(II) spot test

<table>
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<th>Method</th>
<th>Product</th>
<th>Yield %</th>
<th>Cu(II) spot test</th>
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SCHEME 1: Solvent-free pyrophosphate coupling in a ball mill reported in reference 1.

References

Acknowledgements
We are grateful to Richard Murray, and Cormac Miller for technical support.

FIGURE 1: a. Retsch® vibration ball mill MM400; b. Copper-c Zirconia-lined; d. degraded steel- reaction vessel.

FIGURE 2: 1H-NMR of crude reaction mixture (SCHEME 3: iv)

FIGURE 3: 31P-NMR of purified AppsA (SCHEME 3: 11)

FIGURE 4: 1H-NMR of purified AppsA (SCHEME 3: 11)