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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 MeOH, DMSO 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 solvothermal nucleoside chemistry as recently demonstrated by rapid, atom-economic pyrophosphate coupling (SCHEME 1).1 In the absence of solubility issues, phosphates 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 monoester (AMP-M; 1) with 5’-di-o-hydroxyethyl monophosphorothiolate in a zinc-iron-lined vessel (FIGURE 1c).

FIGURE 1. A. Zirconia vibration ball mill MM400; B. Copper; C. Zincia-lined; D. Degraged steel - reaction vessel.

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.2 Typically, reactive CuI is either generated in situ from a CuI(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-diketone formation.3 Based upon these observations, we have investigated “click” cycloaddition reactions between 5’-azido-5’-deoxythymidine (4) and mono-5(5a-c) or bis-5(5d) N-propargylamide-substituted azobenzenes4 under different reaction conditions (SCHEME 2). In solution, high to quantitative yields were achieved using 5 mol% CuI(II) in the presence of TBTA (TABLE: Method A). “Click” reactions using the monopropargylamines were also effected in the presence of added cuprous salts by the application of ball milling in metallic copper core reaction vessels; high speed vibration ball milling (HSVBM) using a 33/23 (2.38 mm) diameter copper ball (62 mg) for 1 hour overnight in the presence of ethyl acetate lead to complete consumption of the 5’-azido nucleoside with clean conversion to the corresponding 1,3-diketone without contamination by copper (TABLE: Method B). In contrast, more rapid reaction was induced in a more capacious copper vessel using higher energy inputs 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 (R(SP2)3O) are susceptible to hydrolytic P-S bond cleavage especially under acidic conditions5 and therefore are incompatible with the prolonged reaction times typically required for solution-phase pyrophosphate coupling under Khorana conditions.6 CDI-mediated coupling is more rapid and was used by Patel and Eckstein to activate 5’-thioadenosine-5’-phosphothiolate monomer in order to effect coupling with [PO3H2][Bu4N]+ but the corresponding triphosphate was isolated in low yield.8 5’-Thioadenosine 5’-phosphorothiolate (P–5’)-adenosine (AppsA -11) was prepared over a series of steps (SCHEME 3). Attempted nucleophilic displacement of chloride from 5’-Thioadenosine 5’-phosphorothiolate (7) using sulfur nucleophiles in a steel vessel (FIGURE 1d) resulted in its corrosion and product contamination as reported by other workers.18 In contrast, reaction of p-anisyl-mercaptan in zirconia-lined vessels (FIGURE 1c) gave the thioether (8) cleanly. One pot deprotection and disulfide exchange enabled isolation of the activated disulfide (9) in good yield. For the key pyrophosphate coupling, initial Michaelis-Atkinsov reaction of 9 was performed in the presence of excess silating agent.10 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 45% yield (from 9) and characterised by NMR (FIGURES 3 and 4).

SCHEME 3. Reagents and conditions: i) p-anisyl-mercaptan (3 eq.), base (9 eq.), VBM at 30 Hz, 1 h, 87%; ii) 2,2-dithiobis(5-methylpyridine) (2 eq.), acid, 2 h, 75%; iii) a) CH2Cl2, TBSMA (41), RT, 30 min; b) TMSO (P=1 eq.), RT, 30 min; iv) AMP-M (1-1.5 eq.), Me2SO,CH2=CH2O (1.5 eq.), tert-butylamine (2.1 eq.), H2O (12 eq.), zincia-lined vessel, VBM at 30 Hz, 1.5 h, 40%.

Summary

In this study we have demonstrated the capacity of ball-milling to facilitate nucleophilic substitution upon a halonucleoside in the absence of DMP and synthesised a novel pyrophosphorothiolate-linked dinucleoside (AppsA) via an acid-sensitive phosphorothiolate monoester using ball milling to effect the key one-pot hydrolytic desilylation / pyrophosphate coupling. As 5’-linked structures are substrates for ligation, this may provide a more efficient (biochemical) route for 5’-phosphorothiolate linkages than that via the monoester.16

References