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COMMUNICATION

Rapid synthesis of nucleotide pyrophosphate linkages in a ball mill†

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Using a ball mill, rapid, atom-economic coupling between adenosine-5′-phosphoromorpholidate and phosphorylated ribose derivatives as their sodium or barium salts was achieved. Facile purification by reversed-phase HPLC enabled product isolation within hours.

Nucleotides bearing pyrophosphate linkages are ubiquitous in biological information and energy transduction systems[1] and have therefore long been the target of chemical synthesis.[2]

Currently, phosphoromorpholidates are widely used in pyrophosphate synthesis due to the balance between their electrophilicity towards phosphate anions and their hydrolytic lability.[3] Typically, such coupling reactions are performed in anhydrous pyridine, DMF or formamide.[4] In order to render polyanionic phosphates soluble in these solvents, extensive cation exchange (ultimately to give the corresponding trialkylammonium salts) and predrying is required. Coupling proceeds in poor to fair yields over extended times (16 h to 6 days) and hydrolysis of the phosphoromorpholidate group over this period often renders the subsequent purification problematic due to the presence of isoelectric homodimers.

In attempting to overcome these issues, a wide variety of coupling chemistries has been described including direct dehydration methods and *in situ* activation *via* phosphoroimidazolides or mixed anhydrides.[5] Perhaps most successful in ameliorating purification issues has been reaction of activated intermediates with solid-bound substrates.[6] However, typically large reagent excesses and anhydrous conditions are still required.

Mechanochemical mixing of solids with disparate solubility properties is well established and has found wide employment in materials science[7] but only in the last two decades has the application of mechanochemistry to organic synthesis been more rigorously explored.[8] In particular, the synthesis of nucleoside and nucleotide derivatives using ball milling remains undeveloped.[9]

Here, we report a methodology for the synthesis of both symmetric and non-symmetric nucleoside polyphosphates in fair to good yields using a vibration ball mill without extensive pre-treatment of the substrates (Scheme 1). Following previous studies upon nucleoside protection,[9a] all reactions were performed using a 25 mL stainless steel jar containing a single 15.0 mm stainless steel ball. The jar was charged with equimolar amounts of adenosine-5′-phosphoromorpholidate (**1**; upto 0.14 mmol) and a phosphorylated ribose derivative (**2a–f**). In addition, magnesium chloride hexahydrate, 1*H*-tetrazole and water were all found to be necessary to give a complete reaction and suppress the side-reactions of the AMP-morpholidate. The jar containing these reagents was shaken at 30 Hz for 90 min, allowed to cool to room temperature and the reaction mixtures suspended in water prior to analysis and then purification using reversed-phase HPLC. In all cases, essentially complete consumption of the phosphoromorpholidate was observed with up to 87% conversion to the desired product, *e.g.*, Ap_2dT (**3d**) (Fig. 1).

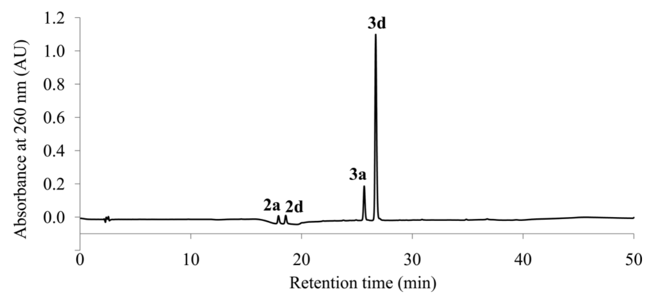

Fig. 1 Analytical C18 RP-HPLC chromatogram of the crude reaction mixture of Ap_2dT (**3d**) synthesis (entry 4). AMP (**2a**) arising from hydrolysis of **1**, unreacted dTMP (**2d**), and Ap_2A (**3a**) are also visible.

In the absence of acid promoters,[10] no consumption of **1** was observed. Addition of Mg(II)[11] alone enhanced the rate of coupling between **1** and AMP (**2a**) and gave clean conversion to **3a**. However, after 150 min, less than 50% conversion was observed. In contrast, complete consumption of **1** was observed after 90 min in the presence of excess tetrazole[12] but multiple side-products were apparent. In particular, tetrazole catalysed both the hydrolysis of **1** to **2a** as well as the homo-coupling of **1** (putatively through the 2′- or 3′-hydroxyls). We have previously found that solvent-assisted grinding (often called liquid-assisted grinding, or LAG)[13] can enhance the selectivity of amine acylation[9b] and in this work the addition of water (6 eq) was found to suppress self-coupling of **1**.

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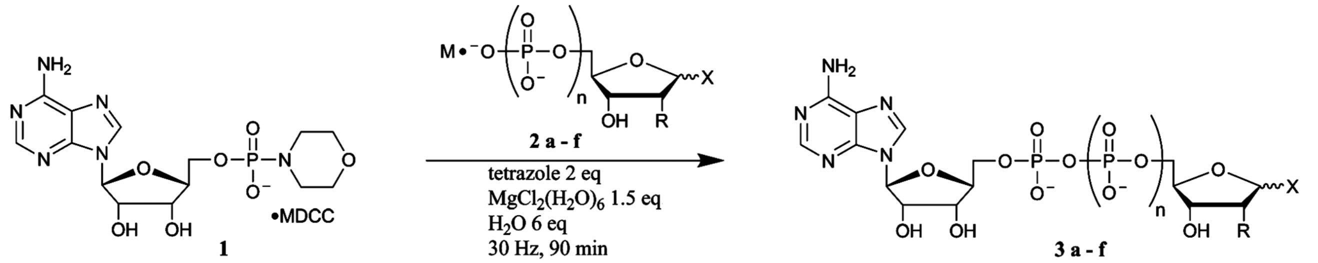

entry	Substrate		M	R	n	X	Product		% Yield HPLC[a]	Isolated[b]
1	**2a**	AMP	$H_{0.9}Na_{1.1}$	OH	1	β-9-*N*-adeninyl	**3a**	Ap_2A	89	75
2	**2b**	ADP	$H_{1.6}Na_{1.4}$	OH	2	β-9-*N*-adeninyl	**3b**	Ap_3A	53	49
3	**2c**	ATP	H_2Na_2	OH	3	β-9-*N*-adeninyl	**3c**	Ap_4A	56	52
4	**2d**	dTMP	Na_2	H	1	β-1-*N*-thyminyl	**3d**	Ap_2dT	74	71
5	**2e**	NMN	H	OH	1	β-3-carbamoylpyridin-1-ium-1-yl	**3e**	NAD^+	68	58
6	**2f**	RMP	Ba	OH	1	α- / β-OH	**3f**	ADPR	52	43[c]

Key MDCC = 4-morpholine-*N*,*N*'-dicyclohexylcarboxamidinium; [a]calculated based upon relative peak areas at A^{260nm}; [b]calculated based upon triethylammonium salts (as defined by ^1H NMR); [c]isolated as a mixture of α- and β-anomers (37:63 respectively).

Scheme 1 Synthesis of pyrophosphate linkages in a ball mill.

Thus, in the presence of $MgCl_2(H_2O)_6$, tetrazole and water, enhanced rates and intermolecular selectivity of the phosphate coupling reaction were observed. Moderate to high yields were observed for the preparation of other dinucleoside polyphosphates (**3b–d**), nicotinamide adenine dinucleotide (NAD^+ - **3e**) and adenosine diphosphate ribose (ADPR – **3f**). Side-products arising from hydrolysis of **1** to AMP (**2a**) accompanied by the production of Ap_2A (**3a**) were also observed at varying levels (12–31%).

The mobile-phase gradients developed for both analytical and preparative scale HPLC enabled the separation of all components and purification of the products could therefore be completed within 150 min. Lyophilisation of the solutions removed both the water and volatile salts to yield the pure products as their triethylammonium salts. Again, this procedure is much faster than typical ion-exchange chromatography using DEAE Sephadex.

In conclusion, we have developed atom-economic methodology for pyrophosphate bond formation using stable, inexpensive and commercially-available reagents which does not require the use of anhydrous, non-volatile and toxic solvents, which is compatible with the synthesis of natural or modified linkages and which enables rapid and facile isolation of pure materials.

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Notes and references

1 *e.g.* A. G. McLennan, L. D. Barnes, G. M. Blackburn, C. Brenner, A. Guranowski, A. D. Miller, J. M. Rovira, P. Rotllan, B. Soria, J. A. Tanner and A. Sillero, *Drug Dev. Res.*, 2001, **52**, 249–259.
2 (*a*) J. Baddiley and A. R. Todd, *J. Chem. Soc.*, 1947, 648–651; (*b*) A. Todd, *Science*, 1958, **127**, 787–792; (*c*) G. M. Blackburn, M.-J. Guo and A. G. McLennan, in *Ap_4A and Other Dinucleoside Polyphosphate*, ed. A. G. McLennan, CRC Press, Boca Raton, 1992.
3 J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, 1961, **83**, 649–658.
4 (*a*) A. Adam and J. G. Moffatt, *J. Am. Chem. Soc.*, 1966, **88**, 838–842; (*b*) J. Lee, H. Churchil, W. B. Choi, J. E. Lynch, F. E. Roberts, R. P. Volante and P. J. Reider, *Chem. Commun.*, 1999, 729–730.
5 (*a*) T. C. Myers, K. Nakamura and J. W. Flesher, *J. Am. Chem. Soc.*, 1963, **85**, 3292–3295; (*b*) I. B. Yanachkov, E. J. Dix, M. I. Yanachkova and G. E. Wright, *Org. Biomol. Chem.*, 2011, **9**, 730–738; (*c*) T. C. Lin and J. M. Fang, *Tetrahedron Lett.*, 2011, **52**, 2232–2234; (*d*) Q. W. Han, B. L. Gaffney and R. A. Jones, *Org. Lett.*, 2006, **8**, 2075–2077.
6 *e.g.* Q. Dai, M. Saikia, N. S. Li, T. Pan and J. A. Piccirilli, *Org. Lett.*, 2009, **11**, 1067–1070.
7 P. Baláž, *Mechanochemistry in Nanoscience and Minerals Engineering*, Springer-Verlag Berlin, Heidelberg, 2008, pp. 1–102.
8 A. Stolle, T. Szuppa, S. E. S. Leonhardt and B. Ondruschka, *Chem. Soc. Rev.*, 2011, **40**, 2317–2329 and references cited therein.
9 (*a*) N. Giri, C. Bowen, J. S. Vyle and S. L. James, *Green Chem.*, 2008, **10**, 627–628; (*b*) F. Ravalico, S. L. James and J. S. Vyle, *Green Chem.*, 2011, **13**, 1778–1783; (*c*) C. Hardacre, H. F. Huang, S. L. James, M. E. Migaud, S. E. Norman and W. R. Pitner, *Chem. Commun.*, 2011, **47**, 5846–5848; (*d*) S. A. Sikchi and P. G. Hultin, *J. Org. Chem.*, 2006, **71**, 5888–5891.
10 R. W. Chambers and H. G. Khorana, *J. Am. Chem. Soc.*, 1958, **80**, 3749–3752.
11 M. Shimazu, K. Shinozuka and H. Sawai, *Tetrahedron Lett.*, 1990, **31**, 235–238.
12 V. Wittmann and C. H. Wong, *J. Org. Chem.*, 1997, **62**, 2144–2147.
13 T. Friščić and W. Jones, *J. Pharm. Pharmacol.*, 2010, **62**, 1547–1559.