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CONVENIENT SYNTHESES OF ADENOSINE 5'-DIPHOSPHATE, ADENOSINE 5'-METHYLENEDIPHOSPHONATE, AND ADENOSINE 5'-TRIPHOSPHATE

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Abstract: Adenosine 5'-tosylate is converted to adenosine 5'-diphosphate (ADP), adenosine 5'-methylenediphosphonate, and adenosine 5'-triphosphate (ATP) in good yields by direct displacement with the appropriate inorganic salt.

Nucleosides are normally phosphorylated by displacement at an activated phosphorus with a nucleophilic sugar hydroxyl.[1-5] The procedure is convenient for synthesis of monophosphate derivatives, but additional displacements or condensations are usually required to prepare common polyphosphorylated compounds such as ADP or ATP. We recently published a procedure for introduction of the pyrophosphate moiety in the synthesis of isoprenoid diphosphates by nucleophilic substitution at carbon.[6,7] This approach has the advantage that the phosphate-containing unit is introduced in a single step, and a common carbon-activated precursor can be used to prepare mono-, di-, or triphosphates. The technique can also be used to synthesize diphosphate analogs with a non-oxygen linkage between P_α and P_β. We now report the conversion of adenosine 5'-tosylate to the corresponding diphosphate (ADP), triphosphate (ATP), and methylenediphosphonate.

The displacements were conducted on 5'-tosylates of adenosine and the 2',3'-*O*-isopropylidene derivative. The nucleophilic monohydrogen tetra-n-butylammonium salts of diphosphate, triphosphate, and methylenediphosphonate were prepared by titration of aqueous solutions of the respective acids with tetra-n-butylammonium hydroxide.[6] Water was removed by lyophilization and the resulting off-white hygroscopic powders were stored at 4°C in a dessicator. These materials are unstable to prolonged storage under these conditions and should be freshly prepared or maintained at -20°C if they are to be kept for longer than a month. Displacements with the salts and 5'-activated nucleosides were carried out in freshly distilled, dry acetonitrile using 0.5 molar excesses of the salts. The products were purified by flash chromatography on cellulose, and the results are summarized in Table I.

Table I. Phosphorylation of Nucleoside Tosylates by Direct Displacement

Nucleoside	Nucleophile	reaction time (h)	yield (%)
2',3'-*O*-Isopropylidene-adenosine 5'-tosylate[a]	$(nBu_4N)_3HP_2O_7$	2	93
Adenosine 5'-tosylate[b]	$(nBu_4N)_3HP_2O_7$	48	74
	$(nBu_4N)_3HPO_3CH_2PO_3$	48	72
	$(nBu_4N)_4HP_3O_{10}$	48	72

[a] Jahn, W. *Chem. Ber.* 1965, 98, 1705 - 1708. [b] Aldrich Chemical Co.

In spite of long reaction times, the yields of ADP, ATP, and adenosine 5'-methylene diphosphonate are good. 2',3'-*O*-Isopropylideneadenosine 5'-tosylate is substantially more reactive toward the diphosphate anion than adenosine 5'-tosylate itself, and the yield for displacement is substantially higher when the hydroxyl moieties are blocked. The origin of the rate enhancement is not known. Perhaps the isopropylidine group induces a preference for a more reactive conformation at C5' or eliminates unproductive hydrogen bonding between the pyrophosphate and the 2'- and 3'-hydroxyls. In early experiments we found that tetraalkyl ammonium salts are much more reactive than trialkyl ammonium derivatives and attribute the differences to reduced nucleophilicity because of hydrogen bonding between the trialkyl ammonium salts and the phosphorus nucleophiles. From a practical viewpoint, however, the gains in reactivity and yield are offset by the extra steps needed to introduce and remove the isopropylidene moiety.

A typical experiment is illustrated by the synthesis of ATP. Adenosine 5'-tosylate (140 mg, 0.33 mmol) was dissolved in 0.2 mL of acetonitrile, 611 mg (0.5 mmol) of *tetra*(tetra-n-butylammonium) hydrogen triphosphate was added, and the resulting viscous solution was allowed to stir under nitrogen at room temperature for 48 h. High concentrations of tosylate (1.5 M)

and salt (2.5 M) were necessary for the reaction to proceed at a reasonable rate.[8] The progress of the reaction was followed by monitoring the changes which occur for the chemical shifts of the aromatic tosylate protons upon displacement. The four line AA'BB' pattern (δ_{AA}' ~ 7.28 ppm, δ_{BB}' ~ 7.68 ppm) for adenosine 5'-tosylate decreased in intensity as the reaction proceeded and was replaced by new AA'BB' signals (δ_{AA}' ~ 7.10 ppm, δ_{BB}' ~ 7.54 ppm) for the tosylate anion. Upon completion, the mixture was diluted with 4 mL of water. Tetra-n-butylammonium was replaced with ammonium by ion exchange chromatography on Dowex AG 50W-X8 (ammonium form, 100-200 mesh, 2 x 19 cm). Two column volumes were collected and lyophilized. The resulting off-white solid was extracted with 2 mL of acetonitrile:100 mM ammonium bicarbonate:concentrated ammonium hydroxide, 7:3:2, and the soluble portion was chromatographed on CF-11 cellulose (2 x 26 cm) with the same mixed solvent. Four mL fractions were collected, and those containing ATP (fractions 40-60) were combined. Acetonitrile was removed by rotary evaporation and water, by lyophilization to yield 129 mg (75%) of a white powder, R_f 0.15 (cellulose, acetonitrile:100 mM ammonium bicarbonate:concentrated ammonium hydroxide, 7:3:2).

In summary, adenosine 5'-diphosphate,[9] adenosine 5'-triphosphate,[10] and adenosine 5'-methylenediphosphonate[11] can be synthesized by direct displacement at the 5'-position of adenosine 5'-tosylate with the appropriate phosphorus-containing nucleophile. These reactions demonstrate that the procedure we originally reported for synthesis of allylic isoprenoid pyrophosphates[6] can be extended to include a variety of non-allylic primary systems. The method provides a convenient synthesis of the methylenediphosphonate analog of ADP[5] and should be a useful general procedure for synthesis of phosphorylated analogs where the bridging oxygen between P_α and P_β has been replaced by another moiety. Experiments are underway to explore the scope of the reaction for synthesis of other biologically important molecules and analogs.

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8. Runs at elevated temperatures to shorten the reaction time gave lower yields.

9. ^{1}H NMR (D_2O, DSS internal standard) 4.45 (5, m), 6.15 (1, d, J = 4.5 Hz), 8.05 (1, s), 8.45 ppm (1, s); ^{31}P NMR (D_2O, H_3PO_4 external standard, ^{1}H decoupled) 5.67 (1, d, J = 21.8 Hz) and 9.92 ppm (1, d, J = 21.8 Hz).

10. ^{1}H NMR (D_2O, DSS internal standard) 4.55 (5, m), 6.15 (1, d, J = 4.5 Hz) 8.10 (1, s), and 8.45 ppm (1, s); ^{31}P NMR (D_2O, H_3PO_4 external standard, ^{1}H decoupled), 5.62 (1, d, J = 19.8 Hz), 10.43 (1, d, J = 19.8), and 21.10 ppm (1, t, J = 19.8 Hz).

11. ^{1}H NMR (D_2O, DSS internal standard) 2.2 (2, t, J = 19.5 Hz), 4.45 (5, m), 6.10 (1, d, J = 4.5 Hz), 8.14 (1, s), and 8.46 ppm (1, s); ^{31}P NMR (D_2O, H_3PO_4 external standard, ^{1}H decoupled) -19.3 (1, d, J = 9.4 Hz) and -13.96 ppm (1, d, J = 9.4 Hz).

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