thus defining the stereochemistry of 8 and accordingly of 5 as *Z*.

While the origin of the *Z* stereoselectivity of these Wittig reactions remains to be determined,[11] the remarkably high *Z* stereoselective synthesis of the allylic oxygenated olefins described herein should find general synthetic applications.

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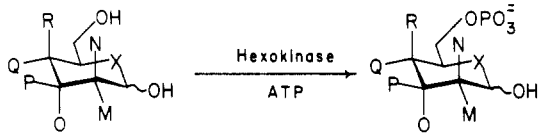
Chemoenzymatic Syntheses of Fluoro Sugar Phosphates and Analogues

Summary: Combined chemical and enzymatic procedures are described for the preparation of fluorinated sugar phosphates and analogues. These derivatives are useful for study of sugar metabolism and for synthesis of pharmacological probes in a number of enzymatic systems utilizing sugars.

Sir: This paper describes studies of a regioselective, enzyme-catalyzed phosphorylation of fluorinated sugars and sugar analogues with heteratoms in the pyranose rings (Scheme I) which provides a combined chemical and enzymatic route to potentially useful pharmacological probes in numerous enzyme systems.

Current studies indicate that fluorinated sugar phosphates or sugar nucleotides in which one of the hydroxyl groups is replaced with the fluorine group can be strong inhibitors of the nonfluorinated species and are of interest as potential pharmaceuticals or pharmacological probes.[1] The inhibitions are due to the difference of C–F and C–OH in reactivity and the similarity of both groups in polarity and bond length.[2] Despite the usefulness of this class of compounds, the synthesis, however, still depends on chemical procedures which require multiple protection and deprotection steps to overcome the problems of regioselectivity.[3] In particular, chemical phosphorylation of fluorinated sugars requires a different protection strategy from that of the nonfluorinated counterparts. As a part of our interest in developing enzymatic routes to this class of compounds, we have surveyed the substrate specificity of yeast hexokinase (E.C. 2.7.1.1) on a variety of fluorinated hexopyranoses and glucose analogues with S or NH in the ring (Scheme I).[4] As shown, each of compounds **a–j** can be accepted as a substrate for the enzyme. Although high specific activity of enzymes used as catalysts in large-scale organic synthesis permits the construction of efficient reactors, low specific activity of enzymes using weak substrates could also be valuable and practical provided the enzymes and the cofactor regeneration system used are inexpensive and stable. In order to illustrate the practicality of the enzymatic preparation of unnatural sugar phosphates using the ATP-requiring hexokinase reactions, we selected a weak substrate and a strong ATP regeneration system based on pyruvate kinase as a catalyst and phosphenolpyruvate as a phosphoryl donor which has excellent stability in solution.[5]

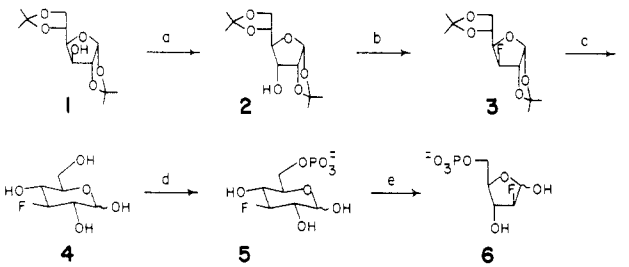
Scheme I[a]



Cmpd	M	N	O	P	Q	R	X	Km (mM)	Vmax (Umg^{-1})
a	OH	H	H	OH	OH	H	O	0.17	300
b	F							0.19	150
c	H	F						0.41	260
d	F	F						0.13	160
e			F	H				82	30
f				F				70	30
g					F			84	30
h					H	F		–	3
i							S	4	15
j							NH	1	30

[a] The substituents other than those for glucose (a) are indicated. 1 U = 1 μmol product formed per min.

Scheme II.[a] Synthesis of 2-Deoxy-2-fluoro-D-arabinose 5-Phosphate (6)



[a] (a) 1. pyridinium dichromate/acetic anhydride, 96%. 2. $NaBH_4$/70% aqueous ethanol, 92%. (b) DAST/CH_2Cl_2/pyridine, 88%. (c) Dowex 50 (H^+)/H_2O, 92%. (d) ATP/hexokinase/phosphoenolpyruvate/pyruvate kinase, 86%. (e) $Pb(OAc)_4/H^+$, 64%.

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The representative preparation of 2-deoxy-2-fluoro-D-arabinose 5-phosphate (**6**) was carried out as shown in Scheme II. 1,2:5,6-Di-*O*-isopropylidene-D-glucofuranose (**1**) was first converted to the D-allose derivative **2**[6] which upon reaction with (diethylamino) sulfur trifluoride (DAST)[7] in methylene chloride and pyridine was transformed to the diisopropylidene derivative **3**. The isopropylidene groups were removed by hydrolysis with Dowex 50 (H^+) in water to yield 3-deoxy-3-fluoro-D-glucose (**4**): mp 114–115 °C (lit.[8] mp 112–113 °C); $[\alpha]_D^{20}$ +6.5° (*c* 1, H_2O) (lit.[1] $[\alpha]$ +6.4°, *c* 1, H_2O). Compound **4** (20 mmol) in aqueous solution (100 mL, pH 7.0) was phosphorylated at the 6 position to give **5** by ATP (0.2 mmol) catalyzed by yeast hexokinase (246 units, immobilized in 5 mL of polyacrylamide gels) coupled with a cofactor regeneration system containing immobilized pyruvate kinase (315 units, 3 mL gels) and phosphoenolpyruvate (22 mmol).[5] The conditions are essentially the same as those reported previously.[9] HPLC and enzyme analyses[10] indicated that the reaction was complete in 7.5 days. Compound **5** was isolated as a barium salt as described previously for the preparation of glucose 6-phosphate.[9] Enzymatic analysis indicated that 7.9 g of the product contains 87% of compound **5** as monobarium salts. At the conclusion of the reaction, each of the recovered enzyme activities was about 90% of their original activities and the turnover number for ATP was 100. Further oxidation of **5** (the barium ions were removed by treatment with Dowex 50) with 1.6 equiv of lead tetraacetate in acetic acid[11] gave compound **6** which was isolated in 62% yield as a sodium salt: $[\alpha]^{25}_D$ −72° (*c* 1.2, H_2O); ^1H NMR (90 MHz) of α form, δ (D_2O) 4.9 (d, $J_{1,2} = J_{2,3} = 0$, $J_{2,F} = 68$ Hz, H_2), 5.3 (d, 1 H, $J_{1,2} = 0$, $J_{1,F} = 9.5$ Hz, H_1); β form, 4.8 (m, $J_{2,F} = 68$ Hz, H_2), 5.2 (q, 1 H, $J_{1,2} = 4$ Hz, $J_{1,F} = 12$ Hz, H_1); ^{13}C NMR δ 63.1 (d, $J_{5,P} = 8$ Hz, C_5). The coupling patterns indicating the F group attached to C_2 and the P group attached to C_5 are consistent with those expected.

In summary, this study illustrates that the substrate specificity of hexokinase is wider than has been suggested in previous studies. This provides potentially critical information relating to the in vivo metabolic disposition of specific fluorinated sugar analogues. This work also suggested that hexokinase/pyruvate kinase should be useful catalysts in preparative synthesis of fluorinated sugar phosphates and analogues using the substrates shown in Scheme I. The phosphate group at the 5 position of pentoses enhances the attractiveness of these sugars as nucleotide precursors as the phosphate group locks the sugar in the furanose form. This allows the formation of a nucleotide having only the furanose configuration without the use of protecting groups.[12] Also, the phosphate group allows direct formation of a nucleotide eliminating the need for phosphorylation of a nucleoside intermediate. Another interesting point which deserves a brief comment is that the amino sugar **j** (Nojirimycin, a transition-state analogue of glucose which has been used as an antibiotic due to its strong inhibition on glycosidase enzymes)[13] is a reasonably good substrate for hexokinase and the phosphorylated derivative is also a good substrate for glucose-6-phosphate dehydrogenase.[14] One of the major concerns about the design and the use of antibiotic drugs is their effectiveness. The study shown here indicates that Norjirimycin might not be a long-lasting and effective antibiotic because it could be further metabolized and inactivated by enzymes in physiological systems.

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Supplementary Material Available: Experimental details for the preparation of compounds **e**, **j**, and **1-6** and their physical constants (8 pages). Ordering information is given on any current masthead page.

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An Efficient Method for the Generation of *N*-Methylnitrones

Summary: *N*-Methylnitrones can be generated in good-to-excellent yields from aldehydes and ketones with a stoichiometric amount of *N*-methyl-*N*,*O*-bis(trimethylsilyl)hydroxylamine under very mild conditions and their formation, involving a bimolecular push–pull type mechanism, is discussed.

Sir: For our studies in the synthesis of natural products, we required a method to generate nitrones under very mild conditions[1] and to subsequently carry out nitrone–alkene cycloadditions in situ.[2] Herein, we report an extremely efficient procedure to prepare a variety of *N*-methylnitrones from *N*-methyl-*N*,*O*-bis(trimethylsilyl)hydroxylamine (**1**)[3] and aldehydes or ketones (Scheme I).

A typical procedure for the synthesis of *N*-methylnitrones is as follows. Treatment of benzaldehyde with a stoichiometric amount of **1** in benzene at 50 °C for 24

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