

**Control of Phosphoryl Migratory Trans-Esterifications Allows
Regioselective Access to Sugar Phosphates**

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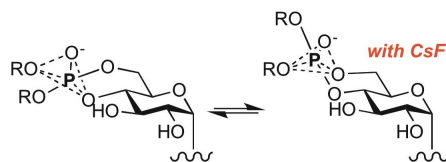
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ABSTRACT



Phosphate esters in polyhydroxylated systems are normally blighted by uncontrolled migration under a variety of reaction conditions. Cesium fluoride is demonstrated as a reagent to control migration of primary phosphates during trans-esterifications. This allows easy exchange of phosphoryl protecting groups enabling enhanced synthetic strategic flexibility and regioselective phosphate installation. Mechanistic analysis suggests that a fluoride-induced extended solvent sphere modulates steric bulk at phosphorus to favor the primary position.

Phosphorylated carbohydrates are prevalent in nature.¹ Their central role in metabolism and signaling make them an important target for the synthetic chemist. However, carbohydrates (and inositols) have an inherent structural complexity that makes their selective phosphorylation difficult.² Installation of phosphoryl groups usually requires multistep sequences that use protecting groups, which results in poor overall yields. Since the high polarity of unprotected phosphate groups can make conventional purification difficult,³ they are typically also

protected to simplify handling. However, this strategy can cause further problems with orthogonality to other functional groups.^{2,4}

In particular, the migration and cyclization of the phosphate group is a common problem requiring special attention.² Neighboring hydroxyls, especially those in a vicinal position, are well placed to attack the phosphorus centre resulting in various side reactions. While protecting groups can lessen this problem, later deprotections may still expose neighboring hydroxyls to conditions favoring phosphate cyclization and migration, resulting in mixtures of polar compounds that can be extremely difficult to purify. For some compounds, catalysts⁵ can offer elegant,

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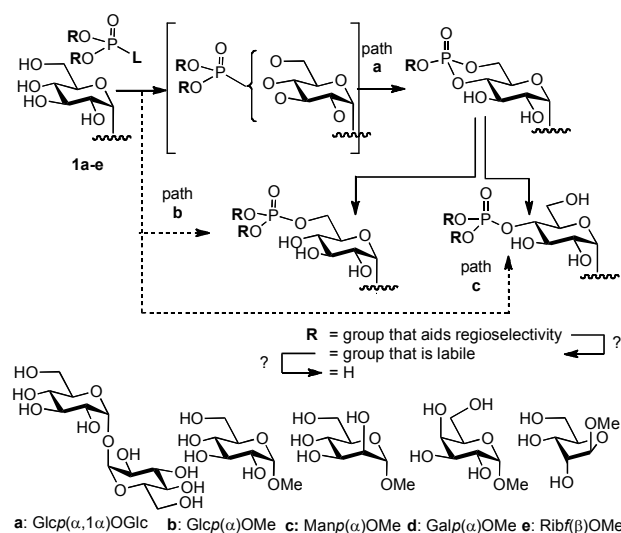
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selective phosphorylations. However, for the majority of carbohydrates and inositols, phosphate migrations remain a major problem that can only be alleviated by careful planning of the synthesis.

We sought conditions to access cyclic-phosphate intermediates, the ‘opening’ of which could parallel the well-exploited 4,6-*O*-benzylidene ring openings where the choice of reagent allows excellent regioselective control.⁶ Under acid catalysis, electronic control dictates protonation leading to the 6-ether, but bulky Lewis acids furnish the 4-ether, via sterically more favourable 6-*O* activation. For phosphates, the steric requirements of the implicated penta-coordinate intermediates might allow similarly good electronic or solvent-mediated control.

Scheme 1. Strategies for regioselective sugar phosphorylation.

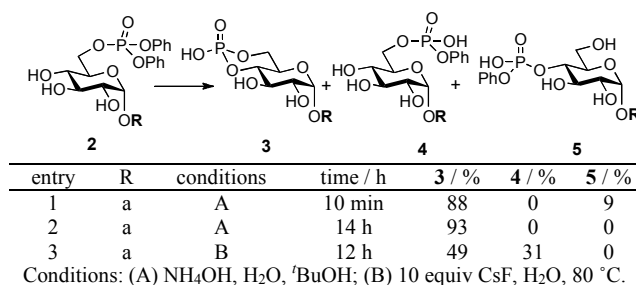


This suggested a phosphorylation strategy with a bis-protected phosphoryl unit followed by cyclization and controlled opening (Scheme 1, route a). If combined with transesterification this could allow ready manipulation of phosphoryl substituents. Such control of migration would also allow potential for regioconvergence from an initially poorly regioselective phosphorylation.

Trehalose, the α,α-1,1-non-reducing dimer of D-glucose, has a complex polyhydroxylated structure that makes it a particularly excellent test substrate. Its 6-*O*-phosphate has been previously synthesized using chemical (POCl₃,⁷ sodium phosphate⁸) or enzymatic

methods.⁹ We have previously shown that diphenyl chlorophosphate can be used to regioselectively install phenyl-protected phosphate groups onto trehalose.¹⁰

Scheme 2. Attempted direct –OPh → –OH deprotection



Direct hydrolytic PhO → OH exchange in **2a** was tested first (Scheme 2), initially with basic hydrolysis.¹¹ On exposure of **2a** to ammonium hydroxide, cyclic phosphate **3a** was produced in 88% yield within 10 minutes (Entry 1); with short reaction times, 9% of the partially deprotected, but migrated, 4-*O*-phosphate **5a** was also isolated. None of the directly deprotected 6-*O*-phosphate was observed. Over longer reaction times, **5** was not isolated and **3a** was the sole product (Entry 2). Importantly, use of CsF¹² (Entry 3) hinted at potential for migration that might be exploited later. While phosphate cyclization remained the predominant pathway (**3** in 49% yield), these conditions also gave **4a**, which crucially, had retained the phosphoryl group at the *O*-6-position.

These results may be rationalized *via* an entropically-favoured intramolecular cyclization giving cyclic ester **6** (Scheme 3).¹³ Hydroxyl ion attack gives a pentacoordinate intermediate (PI₂) leading to three possible products (**3**, **4**, or **5**). Of these, the diester **3** exists in deprotonated form and the repulsive effects of this charge are the source of extreme hydrolytic stability.^{1,14} Thus, nucleophilic attack on **3** is disfavoured and its formation is irreversible, while **4** and **5** are still inclined to cyclization and so are formed reversibly; over time the cyclic phosphate **3** is the major product.

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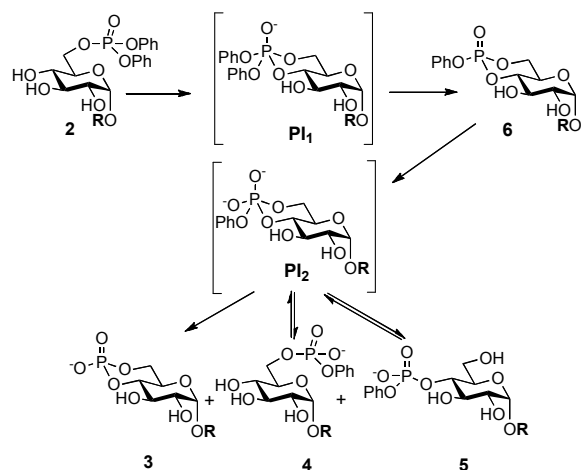
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Scheme 3. ArO \rightarrow OH exchange during hydrolysis leads to irreversible entry into a charged cyclic phosphate manifold.

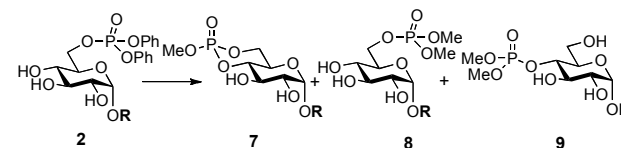


We reasoned that OPh \rightarrow OR transesterification would produce a cyclic phosphate intermediate that would not be charged and could therefore be formed reversibly. Hence, we tested reactions using MeOH as solvent (Scheme 4). Initial investigations under strongly basic conditions resulted in B_{Al2}/S_N2 -type solvolysis of exchanged intermediates leading to mono-deprotection and entry into the unwanted charged cyclic phosphate (**3**) manifold. Under milder conditions methyl loss did not occur; we were pleased to see that uncharged cyclic phosphate **7a** was quickly formed and, over time, reacted further to give **8a** and **9a**. Thus, triethylamine (Entry 1) allowed transesterification without concomitant deprotection albeit with substantial migration from *O*-6 to *O*-4 (63% r.r. *O*-4 **9a**). In the presence of CsF (Entries 2,3), *O*-4 migration was reduced in a manner dependent on the amount of CsF used, up to 10 equivalents, which gave 65% r.r. of *O*-6 **8a**. Importantly, these results demonstrated entry into a manifold primed for migration and suggested that regioselectivity could be modulated.

A striking feature of these reactions was the reduced propensity for *O*-4 migration in the presence of CsF. The reasons for these altered migratory properties were investigated (Scheme 4). First, other fluoride salts with increasing organic solubility (Entries 4 \rightarrow 3 \rightarrow 5) reduced migration to *O*-4. Similarly, increasing solvent proticity¹⁵ (Entries 6 \rightarrow 7 \rightarrow 3 \rightarrow 8) also decreased migration. Third, the reaction was found to be essentially insensitive to pH (Entries 9 and 10) and temperature (Entry 11). Finally, with the different counter-anion Cl⁻ (Entries 12, 13) **3a** was formed exclusively, presumably through Krapcho-type demethylation.¹⁶ Notably, fluorophosphates (potentially isolable compounds¹⁷) were not detected in

any reactions, ruling out direct fluoride participation (see SI for control reactions). It is worth noting that over longer reaction times (Entry 14), further migration to *O*-2 and *O*-3 was observed.

Scheme 4. Conditions for controlled transesterification.



R	conditions	yield ^a %	rr ^b 8:9/ %	other products
1	a NEt ₃ (1 eq), MeOH, 3h	54	37 63	7a (22%)
2	a CsF (2.5 eq), MeOH	91	58 42	-
3	a CsF (10 eq), MeOH	94	65 35	-
4	a KF (10 eq), MeOH	93	61 39	-
5	a TBAF (10 eq), MeOH	51	85 15	3a (24%)
6	a CsF (10 eq), DMF : MeOH (1:1)	59	55 45	3a (22%)
7	a CsF (10 eq), ^t BuOH : MeOH (1:1)	62	59 41	3a (11%)
8 ^c	a CsF (10 eq), 1 H ₂ O : 3 ^t BuOH ^c	31	100 ^c 0 ^c	3a (49%)
9	a CsF, NH ₃ , MeOH, pH 13	92	64 36	-
10	a CsF, HCl, MeOH, pH 2	93	68 34	-
11	a CsF (10 eq), MeOH, 50 °C	90	63 37	-
12	a CsCl (10 eq), MeOH	0	0 0	3a (82%)
13	a MgCl ₂ (10 eq), MeOH	0	0 0	3a (85%)
14	a Na ₂ CO ₃ (2.5 eq), DCM : MeOH (4:1), 80 h	34	32 68	^d
15 ^e	a Ionic liquid : MeOH (1:1), 18h, NEt ₃ (4 eq) ^e	57	100 0	-

Reactions were performed under reflux for 16 h unless otherwise noted. (a) combined yield (b) regioselectivity ratio normalized as %, determined by ¹H NMR of crude mixture and HPLC (c) from Table 1 giving **4** and **5** (d) minor *O*-2,3 migration products (e) conducted in dimethylimidazolium dimethylphosphate and per-*O*-acetylated to aid purification.

These studies revealed that we were able to tune regioselective ratios from 68% in favour of one regioisomer **9** to 100% of the other **8** using appropriate conditions. To test the scope of this strategy, we applied CsF-mediated transesterification to other biologically relevant carbohydrates (Scheme 5). The required starting diphenylphosphates were synthesized in an analogous manner to that used for **2a**.¹⁰ Pleasingly, subsequent transesterifications for **2b-d** all proved successful, indeed more so than for **2a**. Thus, glucoside **2b** gave the *O*-6-phosphate **8b** as the major product in 72%. Similarly, mannoside **2c** furnished the desired *O*-6-phosphate **8c** in an excellent 84% yield. For galactoside **2d**, the initial cyclization was slower (presumably due to the more hindered axial position of OH-4) and the reaction required 40 h for completion. Migratory ring-opening exclusively

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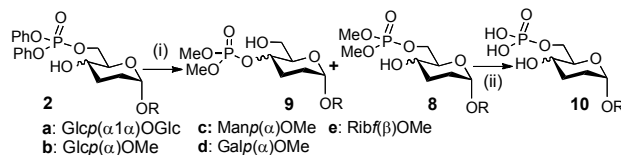
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Gorla, F.; Ringeisen, U.; Rüedi, P. *Helv. Chim. Acta*, **2004**, *87*, 2629–2661 and references therein for preparation isolation and even reaction in alcoholic solvents of fluorophosphates.

yielded the *O*-6-phosphate **8d** in 96%. Riboside **2e** was also successful, (**8e**, 96%); this proceeded *via* a mixed rather than cyclic ester intermediate (see SI for details). Deprotections (Scheme 5) of these dimethylphosphates **8a-e** were achieved with TMSI¹⁸ in excellent yields of greater than 85% for all.

Scheme 5. Transformations for Table 3



Conditions: (i) CsF (10 equiv.), MeOH, reflux; (ii) TMSI, dioxane, 15 min, RT.

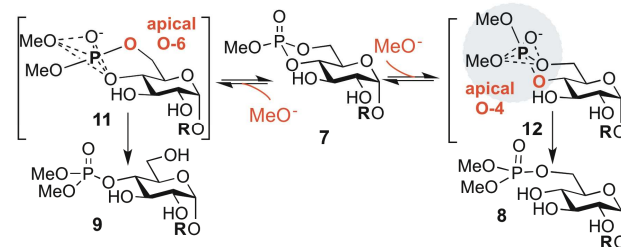
entry	starting 2	time / h	yield 9 / % ^a	yield 8 / % ^a	yield 10 / % ^b
1	a	16	33	61	87
2	b	16	18	72	88
3	c	16	5	84	86
4	d	40	0	96	92
5	e	16	0	96	87 ^c

(a) isolated from step (i); (b) isolated from step (ii); (c) *O*-1 epimer seen.

The protocol we describe allows control of regiochemistry during phosphoryl migrations, which occur *via* a cyclic intermediate¹³ with outcome dictated by a pentacoordinate P centre (Scheme 6).¹⁹ A kinetic preference for migration to OH-4 (cf Scheme 4, Entry 14), observed previously,^{20,21} can be explained by apicophilicity of the most electronegative, primary OH-6 in **11** (see SI for discussion),^{20,22,23} extrusion of the longer P–O(6) bond would be more facile giving **9**. However, sterics may override electronics.²² F[−] ions interact strongly with hydroxyls²⁴ and create H-bonding networks within solvent²⁵ (described as “structure markers” that increase solvent ordering²⁶) particularly in methanolic solutions of CsF.^{27,28} This suggests that F-mediated H-bonding may

create a large solvation sphere around the charged phosphoryl group (**12**, Scheme 6).

Scheme 6.



The increased bulk at OH-4 would then overcome the apicophilicity of OH-6. This effect would be more pronounced in D-galactosides and is consistent with the trend seen for **2d**→**8d** (Table 3, Entry 4). A similar unusual role for CsF in reaction selectivity has also been noted previously in Michael additions.²⁹ Based on this analysis, a reaction was performed in an ionic liquid (Scheme 4, Entry 15) that possesses a highly ordered long-range structure³⁰ and therefore similarly large solvation spheres. We chose a dimethylphosphate derived ionic liquid to prevent interference from the anion.³¹ Under reflux in a 1:1 ratio with methanol and 4 equiv. of NEt₃ as base, the reaction gave a high regioselective ratio >98% for **8a**, consistent with the solvent sphere hypothesis.

In this paper, we have demonstrated methodology that can offer regiocontrol of phosphate migrations. This now enables phosphate transesterification and substituent exchange with all of the associated synthetic strategic advantages. Crucially, exchange of protecting groups allows the introduction of phosphates through a bulky phosphorylating agent prior to alteration to something with orthogonality that allows removal under conditions compatible with other functionality. We believe that this approach will prove useful for installation of the key sugar modification of phosphorylation and may prove applicable to other vital modifications such as sulfation.

Supporting Information Available: This material is available free of charge via the Internet at <http://pubs.acs.org>.

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