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A Fluorous Phosphate Protecting Group with Applications to Carbohydrate Synthesis

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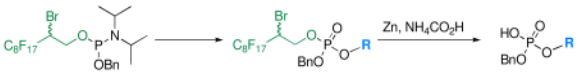
Abstract



The first fluorous protecting group for phosphate is reported. This group can be used as a facile tag for purification and be removed under mild reducing conditions using zinc and ammonium formate. Synthesis of a disaccharide from *Leishmania* using this fluorous protecting group demonstrated the group's stability to the acidic conditions necessary for glycosylation as well as its orthogonality to several other common protecting groups.

Phosphate groups are a common motif in a range of bioactive molecules. Phosphodiester bonds make up the backbone of nucleic acids and phosphate groups are an important part of phospholipids.[1] Phosphorylation is a key modification for numerous proteins and many complex carbohydrates found on the cell surface are phosphorylated. [2] Consequently, the chemistry of phosphate has received a lot of attention. One of the central problems in phosphate chemistry is the protecting group. The phosphate itself is acidic and charged at neutral pH and therefore difficult to carry through and purify by standard organic synthetic methods. Numerous protecting group strategies have been developed for the protection of the phosphate group, most of which have been used in the synthesis of nucleotides, especially on solid-phase. [3] Given the growing interest in fluorous-assisted synthesis[4] and our own interest in the synthesis of phosphate-containing complex carbohydrates, we were intrigued by the possibility of combining a protecting group for phosphate with a fluorous tag for easy purification using fluorous solid phase extraction (FSPE)[5] of the protected compound.

Many fluorous versions of protecting groups have been developed for a variety of functional groups. Our group has used fluorous tags as a handle for purification[6] and also has shown that these fluorous tags/protecting groups could be used to directly array compounds for screening.[7] However, surprisingly no fluorous protecting group for phosphate has yet been reported. Fluorous groups for the temporary tagging of the hydroxyls or permanent tagging of the phosphates of nucleotides have been reported in the context of nucleic acid synthesis to assist separation and some of these fluorous tags have been commercialized.[8] However, since the tags for phosphates cannot be removed, they cannot serve a dual purpose also as a protecting group. We envisioned fluorous protecting groups for phosphates that could function as tags, but also could be removed under mild conditions when necessary, would be

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Supporting Information **Available** Experimental procedures and NMR spectra for all new compounds.

useful in the synthesis of phosphate-containing molecules (Figure 1), allowing both easy purification and a handle for microarray formation. Herein we report the design and synthesis of the first fluorous protecting group for phosphate and demonstrate its use in carbohydrate synthesis.

In the search for a fluorous protecting group for phosphate, 3-(perfluorooctyl)propanol—which contains a C_8F_{17} moiety with a simple three carbon alkyl linker and is commercially available—was a natural starting point. If this fluorous alkyl alcohol could be readily added and removed from a phosphate, it could serve as a protecting group. To test the reactivity of this fluorous alcohol, a model study using dibenzyl 3-(perfluorooctyl)propyl phosphate was initiated. The benzyl groups on the phosphate ideally would serve as the same sort of "permanent" protecting group often used on the hydroxyls of carbohydrates that is removed by hydrogenolysis only at the very end of a synthesis. Generally alkyl protecting groups of phosphates are removed using small nucleophiles.[9] Unfortunately, various nucleophiles such as azide or iodide served only to remove one of the benzyl groups in quantitative yield; the fluorous alcohol largely remained put.

In the continued search for a fluorous protecting group for phosphate that could be easily removed under conditions in which the benzyl phosphate was stable, a haloethyl ester of phosphate caught our attention. These haloethyl groups can generally be removed under mild reducing conditions. Fluorous bromo-alcohol **1**, first reported in 1984,[10] had shown use as a carbamate-type protecting group for amines associated with carbohydrate and peptide structures and could be deprotected using $Zn/Ac_2O/Et_3N$ to provide an *N*-acetyl.[11] We reasoned that this fluorous alcohol **1** with a bromide at the β-position could potentially be suitable for phosphate protection if conditions for its easy removal could be found. However, a concern was the extra stereogenic center of the haloalkyl group, coupled with the stereogenicity of the phosphate ester with a benzyl and a carbohydrate substituent and the chirality inherent in sugars; the resulting diastereomers could make separations and structure elucidation challenging enough to render the fluorous phosphate protecting group more trouble than it was worth.

To first test the relative stability of the fluorous haloalkyl group and the benzyl group on phosphate, a simple dibenzyl phosphate was made using standard phosphoramidite chemistry (Scheme 1). The fluorous bromo-alcohol **1** was coupled with dibenzyl phosphoramidite **2** in the presence of tetrazole to yield phosphite **3**, which was then oxidized to phosphate **4** using *m*-chloroperoxybenzoic acid (*m*-CPBA) in 98% yield for two steps after FSPE purification. Various conditions were tested to remove the fluorous protecting group, including $Zn/NH_4HCO_2/CH_3OH$, Zn/HOAc/THF, and $Pd/C/CH_3OH/NH_4HCO_2$.[12] The reaction was monitored by TLC and ^{31}P NMR. All of these conditions successfully removed the fluorous group on **4**, yielding the desired phosphate **5**, without removal of either benzyl group. The Zn/NH_4HCO_2 conditions in methanol provided the fastest and cleanest reaction. Further optimization of the deprotection conditions showed that using Zn/NH_4HCO_2 in CH_3CN/THF (4:1) the reaction could go to completion in 1-2 hours. The resulting ammonium salt of phosphoric acid was purified by a short silica gel column using CH_2Cl_2/CH_3OH as eluent with 1% NH_4OH to remove the small amount of $ZnBr_2$. The fluorous byproduct of the deprotection, the fluorous alkene, has a boiling point of 146-147 °C and could be removed easily by evaporation. The fraction was concentrated, added water, and subjected to lyophilization to give the pure product.

To study the stability of this protecting group under typical acidic and basic conditions used to remove other protecting groups, compound **4** (0.006 M) was treated with 10% trifluoroacetic acid (TFA, 217 equivalents) or 10% piperdine (168 equivalents) in deuterated chloroform and monitored by ^1H NMR. Haloethyl compounds are known to be unstable to

piperdine; our results showed only 24-hour half-life for the group. In contrast, the fluorous haloalkyl protecting group showed a half-life of over 120 hours in the presence of 10% TFA, and no decomposition was found in the presence of 5 equivalents of TsOH after 48 hours (Table 1). Compound **4** itself is stable at room temperature for several months.

Encouraged by these results, we decided to use this protecting group as a protecting group for phosphate monoester on carbohydrate to ascertain its affect on the separation and characterization of the resulting diastereomeric compounds. As before, we used a benzyl group as the second group on the phosphate ester, since the widely-used benzyl group would not make a final deprotection scheme more complicated by addition of another step. This strategy was first tested on a galactose monosaccharide (Scheme 2).

Benzyl phosphoramidite **6**[13] was coupled with fluorous bromo-alcohol **1** in the presence of tetrazole to yield the desired fluorous phosphoramidite **7**. The ^{31}P NMR of **7** reveals two peaks at 149.57 and 149.44; these peaks reflect the diastereomeric nature of this compound based on the presence of two stereogenic centers. The product was then reacted with diisopropylidene galactose **8**[14] and oxidized with t-BuOOH to afford the desired protected phosphate **10** in 90% yield after FSPE purification without the need of a silica gel column. Two sets of closely spaced peaks in the ^{31}P NMR spectrum showed the influence of the newly added stereogenic center from the carbohydrate, theoretically resulting in 4 diastereomers. Even so, the ^{1}H NMR spectrum of the product was still clean and did not show signs of a mixture. The chiral center on the fluorous bromoalkylalcohol does not complicate the proton NMR analysis likely because of the remoteness of this center from the influence of the carbohydrate. However, ^{31}P NMR can be used to assure that the group has actually been successfully added. Removal of the protecting group using $Zn/NH_4HCO_2/CH_3OH$ required 6-8 hours for completion whereas using CH_3CN/THF (4:1) as solvent reduced the reaction time to 1-2 hours. A short silica gel column, followed by concentration and lyophilization, gave the product **11** as the ammonium salt in 80% yield.

We next wanted to probe the robustness of this new fluorous phosphate protecting group to a set of glycosylation/deprotection conditions used in oligosaccharide synthesis by making a disaccharide from *Leishmania*[15] (Scheme 3). To this end, galactose building block **12** was obtained from D-galactose. [16] The benzylidene was opened selectively using Bu_2BOTf and BH_3THF [17] to yield **13** with a free C-6 hydroxyl. Compound **13** was then coupled with fluorous phosphoramidite **7** to yield **14**. At this stage, four closely spaced peaks in the ^{31}P NMR spectrum (δ 139.52, 139.44, 139.33, 139.26) revealed the product as four diastereomers as expected. After oxidation, phosphate **15** was purified by FSPE and obtained as the only product in 79% yield. The ^{31}P NMR spectrum of **15** showed only two close peaks separated by just 0.01ppm at -1.90 and -1.91. This small difference demonstrates that the chemical environment of the phosphorus in the diastereomeric products are close enough to make little difference in the ^{31}P NMR response. The *p*-methoxybenzyl (PMB) group at C-3 of compound **15** was removed with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[18], followed by FSPE purification to afford **16** with a free hydroxyl acceptor in 97% yield. The acceptor was coupled with trichloroacetimidate donor **17**[19] using trimethylsilyltriflate as promoter followed by another FSPE to yield the desired disaccharide **18** in 94% yield. The fluorous protecting group was removed using Zn/NH_4HCOO in CH_3CN/THF in 2 hours to yield the desired disaccharide **19** as the ammonium salt in 74% yield.

In conclusion, a fluorous protecting group for phosphate group was synthesized. Although this new protecting group contains a stereogenic center, this center does not complicate structure elucidation using ^{1}H NMR and in fact adds diagnostic signals in the ^{13}C and ^{31}P NMR spectra. The fluorous phosphate protecting group greatly simplified the purification

through use of FSPE in the synthesis of phosphate-containing compounds. The fluorous bromo-ethanol could be removed easily under mild reducing conditions using zinc and ammonium formate in CH_3CN/THF. The fluorous byproduct has a relatively low boiling point that allows its easy removal under reduced pressure. We are currently probing the utility of this new protecting group for the synthesis of a series of maltose-related phosphates and for solution-phase automated oligosaccharide synthesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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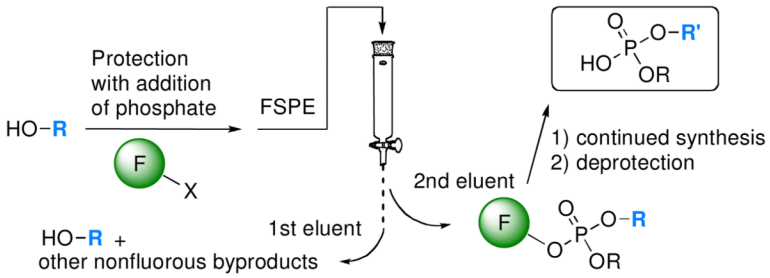
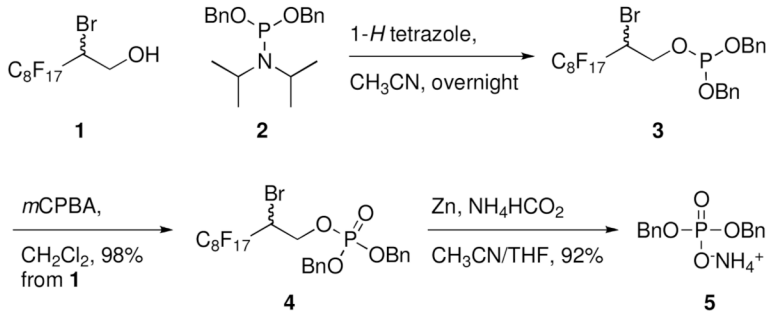
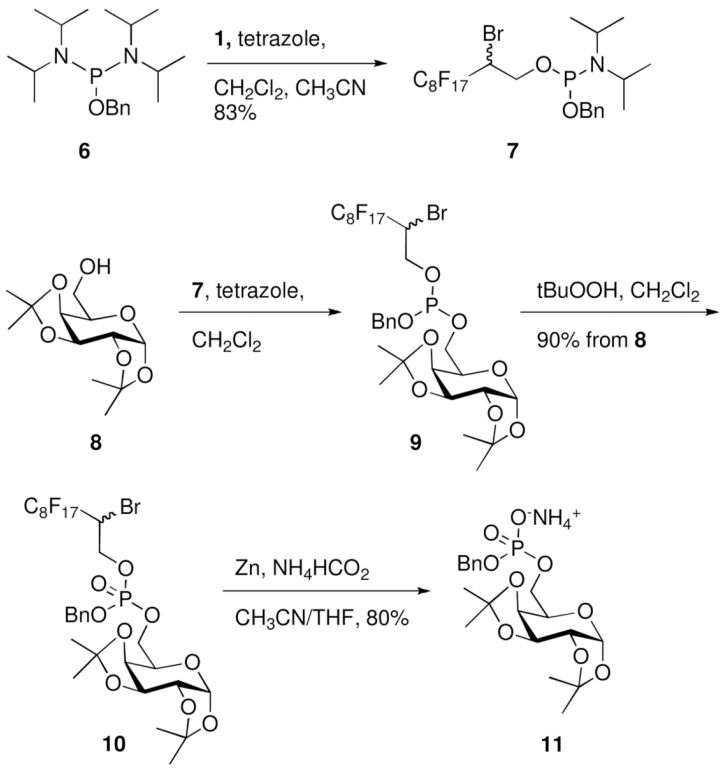


Figure 1.
Concept of the fluorous protecting and tagging group for phosphate.

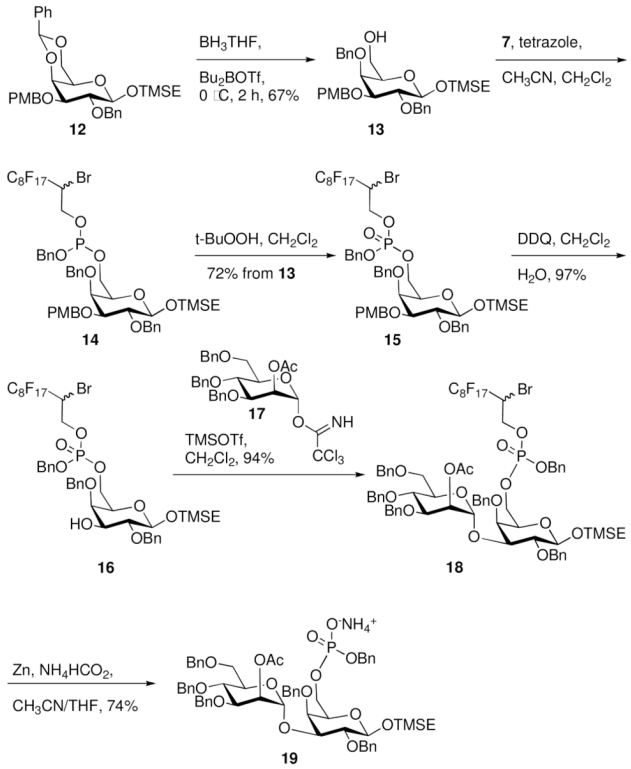


Scheme 1.
Synthesis of fluorous protected dibenzyl phosphate.



Scheme 2.

Fluorous phosphite synthesis, addition to a monosaccharide with formation of the phosphate ester, and deprotection of the fluorous tag.



Scheme 3.
Synthesis of a *Leishmania* disaccharide.

Table 1

Assessment of protecting group stability

	Percentage (%) decomposition of 4						
Time (h)	**0.5**	**12**	**24**	**48**	**72**	**96**	**120**
10% TFA[a]	0	9	17	31	33	42	45
10% piperdine[a]	0	21	50	91	100	--	--
5 equiv TsOH[b]	0	0	0	0	0	--	--

a) in $CDCl_3$;

b) in 9:1 $CDCl_3/CD_3OD$