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A Fluorous Phosphate Protecting Group with Applications to Carbohydrate Synthesis

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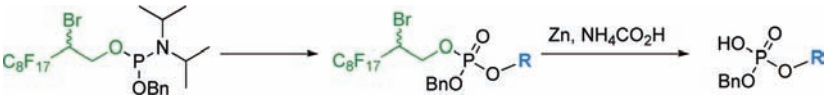
ABSTRACT



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 a 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]

(1) (a) *Nucleic Acids in Chemistry and Biology*, 3rd ed.; Blackburn, G. M., Gait, M. J., Williams, D. M., Eds.; Royal Society of Chemistry: Cambridge, U.K., 2006. (b) Hanahan, D. J. *A Guide to Phospholipid Chemistry*; Oxford University Press: Oxford, U.K., 1997.

(2) (a) Stock, J. B.; Ninfa, A. J.; Stock, A. M. *Microbiol. Rev.* **1989**, *53*, 450. (b) Deutscher, J.; Francke, C.; Postma, P. W. *Microbiol. Mol. Biol. Rev.* **2006**, *70*, 939.

(3) Wuts, P. G. M.; Greene, T. W. *Greene's Protective Groups in Organic Synthesis*, 4th ed.; John Wiley & Sons, Inc: Hoboken, NJ, 2007.

(4) (a) Zhang, W. *Chem. Rev.* **2004**, *104*, 2531. (b) Zhang, W. *Chem. Rev.* **2009**, *109*, 749.

(5) (a) Curran, D. P.; Luo, Z. *J. Am. Chem. Soc.* **1999**, *121*, 9069. (b) Zhang, Q.; Luo, Z.; Curran, D. P. *J. Org. Chem.* **2000**, *65*, 8866. (c) Luo, Z.; Williams, J.; Read, R. W.; Curran, D. P. *J. Org. Chem.* **2001**, *66*, 4261. (d) D. P. Curran. Light Fluorous Chemistry − A User's Guide. In *The Handbook of Fluorous Chemistry*; Gladysz, J. A., Curran, D. P., Horváth, I. T., Eds.; Wiley-VCH: Weinheim, 2004; pp 128−155. (e) Curran, D. P. *Aldrichimica Acta* **2006**, *39*, 3. (f) Zhang, W.; Curran, D. P. *Tetrahedron* **2006**, *62*, 11837.

(6) (a) Jaipuri, F. A.; Pohl, N. L. *Org. Biomol. Chem.* **2008**, *6*, 2686. (b) Pohl, N. L. Automated Solution-Phase Oligosaccharide Synthesis and Carbohydrate Microarrays: Development of Fluorous-Based Tools for Glycomics. In *Chemical Glycobiology*; Chen, X., Halcomb, R., Wang, G. P., Eds.; ACS Symposium Series 990; American Chemical Society: Washington, DC, 2008; pp 272−287. (c) Park, G.; Ko, K. S.; Zakharova, A.; Pohl, N. L. *J. Fluorine Chem.* **2008**, *129*, 978.

(7) (a) Ko, K.-S.; Jaipuri, F. A.; Pohl, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 13162. (b) Chen, G.-S.; Pohl, N. L. *Org. Lett.* **2008**, *10*, 785. (c) Pohl, N. L. *Angew. Chem., Int. Ed.* **2008**, *47*, 3868. (d) Collet, B. Y. M.; Nagashima, T.; Yu, M. S.; Pohl, N. L. B. *J. Fluorine Chem.* **2009**, *130*, 1042. (e) Song, E.-H.; Pohl, N. L. B. *Future Med. Chem.* **2009**, *1*, 889.

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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 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.

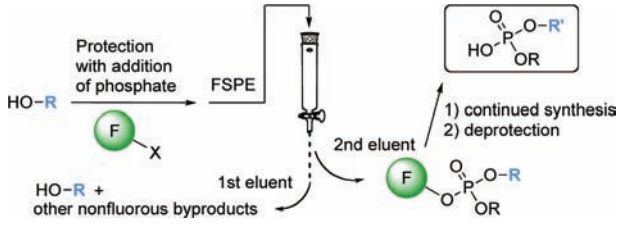
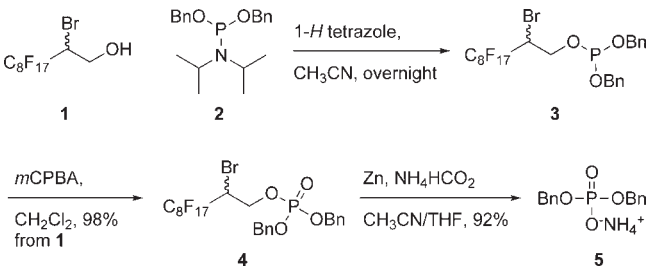


Figure 1. Concept of the fluorous protecting and tagging group for phosphate.

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 yields; the fluorous alcohol largely remained in position.

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.

Scheme 1. Synthesis of Fluorous Protected Dibenzyl Phosphate



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 in 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 by using Zn/NH_4HCO_2 in CH_3CN/THF (4:1) the reaction could go to completion in 1−2 h. The resulting ammonium salt of phosphoric acid was purified by a

(8) (a) Pearson, W. H.; Berry, D. A.; Stoy, P.; Jung, K.-Y.; Sercel, A. D. *J. Org. Chem.* **2005**, *70*, 7114. (b) Tripathi, S.; Misra, K.; Sanghvi, Y. S. *Org. Prep. Proc. Intl.* **2005**, *37*, 257. (c) Beller, C.; Bannwarth, W. *Helv. Chim. Acta* **2005**, *88*, 171. (d) Gupta, A. P.; Will, S. G. Compounds and methods for synthesis and purification of oligodeoxyribonucleotides. PCT Int. Appl. WO 2008077600, 2008.

(9) Holy, A. *Synthesis* **1998**, 381.

(10) Coudures, C.; Pastor, R.; Cambon, A. *J. Fluorine Chem.* **1984**, *24*, 93.

(11) Manzoni, L.; Castelli, R. *Org. Lett.* **2006**, *8*, 955.

(12) (a) Liu, Y.; Lien, I. F. F.; Ruttgaizer, S.; Dove, P.; Taylor, S. D. *Org. Lett.* **2004**, *6*, 209. (b) Ingram, L. J.; Taylor, S. D. *Angew. Chem., Int. Ed.* **2006**, *45*, 3503.

short silica gel column using CH_2Cl_2/CH_3OH as the 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, followed by the addition of water, and subjected to lyophilization to give the pure product.

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	--	--

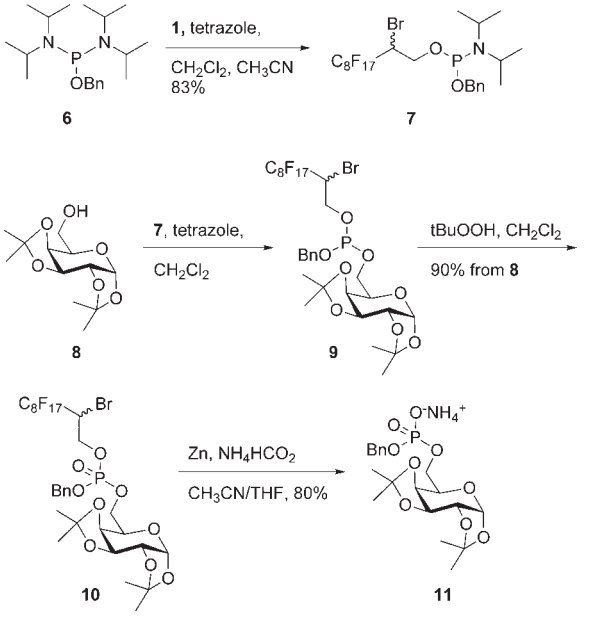
[a] In $CDCl_3$. [b] In 9:1 $CDCl_3/CD_3OD$.

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 equiv) or 10% piperdine (168 equiv) in deuterated chloroform and monitored by ^{1}H NMR. Haloethyl compounds are known to be unstable to piperdine; our results showed only a 24-h half-life for the group. In contrast, the fluorous haloalkyl protecting group showed a half-life of over 120 h in the presence of 10% TFA, and no decomposition was found in the presence of 5 equiv of TsOH after 48 h (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 a phosphate monoester on a 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 the 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 four 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 bromo-alkylalcohol 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 ensure that the group has actually been successfully added. Removal of the protecting group using $Zn/NH_4HCO_2/CH_3OH$ required 6−8 h for completion whereas using CH_3CN/THF (4:1) as the solvent reduced the reaction time to 1−2 h. A short silica gel column, followed by concentration and lyophilization, gave the product **11** as the ammonium salt in 80% yield.

Scheme 2. Fluorous Phosphite Synthesis, Addition to a Monosaccharide with Formation of the Phosphate Ester, and Deprotection of the Fluorous Tag



We next wanted to probe the robustness of this new fluorous phosphate protecting group with 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

(13) Iwashita, M.; Makide, K.; Nonomura, T.; Misumi, Y.; Otani, Y.; Ishida, M.; Taguchi, R.; Tsujimoto, M.; Aoki, J.; Arai, H.; Ohwada, T. *J. Med. Chem.* **2009**, *52*, 5837.

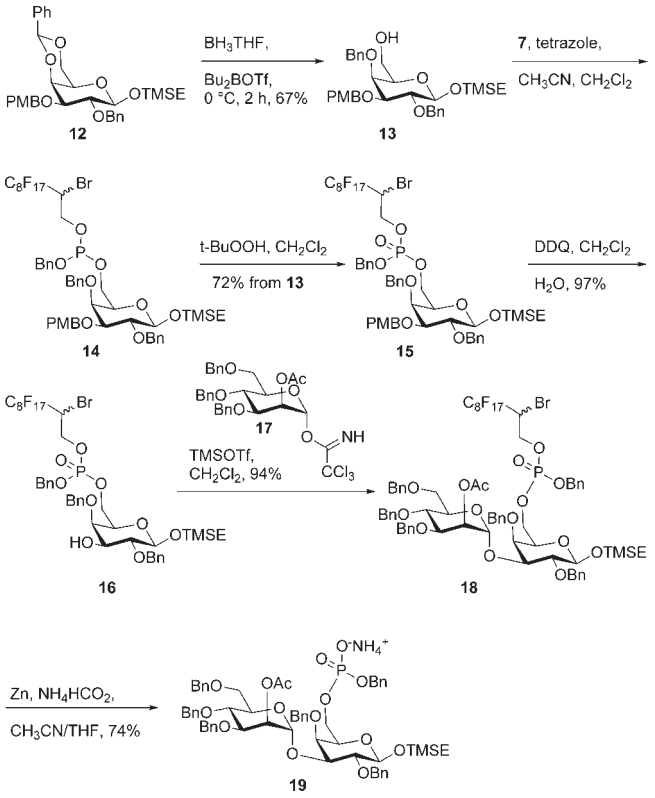
(14) Ferreira, S. B.; Sodero, A. C. R.; Cardoso, M. F. C.; Lima, E. S.; Kaiser, C. R.; Silva, F. P., Jr.; Ferreira, V. F. *J. Med. Chem.* **2010**, *53*, 2364.

(15) Hewitt, M. C.; Seeberger, P. H. *J. Org. Chem.* **2001**, *66*, 4233.

(16) Taylor, J. G.; Li, X.; Oberthuer, M.; Zhu, W.; Kahne, D. E. *J. Am. Chem. Soc.* **2006**, *128*, 15084.

(17) Jiang, L.; Chan, T.-H. *Tetrahedron Lett.* **1998**, *39*, 355.

Scheme 3. Synthesis of a *Leishmania* Disaccharide



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 0.01 ppm at −1.90 and −1.91. This small difference demonstrates that the chemical environments 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 a 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 within 2 h to yield the desired disaccharide **19** as the ammonium salt in 74% yield.

In conclusion, a fluorous protecting group for the phosphate group was synthesized. Although this new protecting group contains a stereogenic center, this center does not complicate structure elucidation by ^{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 the 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.

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Supporting Information Available. Experimental procedures and NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

(18) Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 885.

(19) Watanabe, Y.; Yamamoto, T.; Okazaki, T. *Tetrahedron* **1997**, *53*, 903.