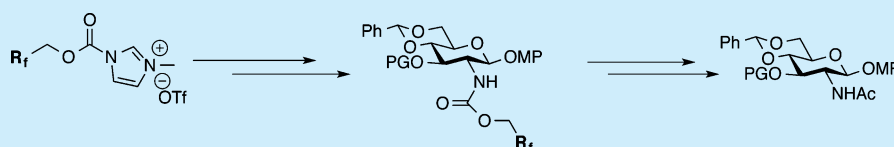


Synthesis of Fluorous Photolabile Aldehyde and Carbamate and Alkyl Carbamate Protecting Groups for Carbohydrate-Associated Amines

Rajarshi Roychoudhury and Nicola L. B. Pohl*

Department of Chemistry, Indiana University, Bloomington, Indiana 47405, United States

S Supporting Information



ABSTRACT: Two new fluorous photolabile-protecting groups (FNBC and FNB) and a new base-labile protecting group (FOC) for the masking of amines are reported. The protecting groups survive a wide range of common reaction conditions used in oligosaccharide synthesis and render the attached molecules amenable to fluorous solid-phase extraction (FSPE). A glycosyl acceptor containing the FNB group is shown to be useful in the synthesis of carbohydrates tagged with free deactivated secondary amines.

The synthesis of oligosaccharides is complex yet often unavoidable given that the isolation of these ubiquitous structures from natural sources, in pure form, is extremely expensive, challenging, and time-consuming.¹ The use of soluble light fluorous tags (those that contain fluorine content equal or less than 40% by molecular weight) has shown promise in simplifying the process of oligosaccharide synthesis; the fluorous-tagged carbohydrate structures can easily be purified from nonfluorous-tagged side products and excess reagents by fluorous solid-phase extraction (FSPE) before continuation of each synthesis cycle.²

Given these advantages, a range of alcohol protecting groups with minimal fluorous content have been designed and applied to carbohydrate synthesis.³ Apart from hydroxyl groups, amines are the next most common functional group found in glycans that requires masking. The *N*-sulfate and *N*-acetyl groups found in natural products are too polar or basic to undergo most protection or other reaction steps in a typical oligosaccharide synthetic sequence and therefore require amine blocking. However, relatively little work has been reported using fluorous variants for nitrogen protection in the realm of carbohydrates. Herein we report the design of a set of three new fluorous amine-protecting groups and demonstrate their tolerance to various standard conditions used in oligosaccharide synthesis.

The most common amine-containing sugars, glucosamine or galactosamine, are often protected by 2,2,2-trichloroethyl chloroformate (TrocCl), allyloxycarbonyl chloride (AllocCl), phthalic anhydride (Phth), and trichloroacetyl chloride (TCACl) to form carbamates or amides when neighboring group participation is needed in the glycosylation step.⁴ After the required oligosaccharide is obtained, the protecting groups are cleaved and then the free amine is acetylated to obtain the final *N*-acetyl or *N*-sulfate moiety. Trichloroacetyl chloride, one of the most common protecting groups used to this end, is

converted to an *N*-acetyl group in one step using AIBN and tributyltin hydride.⁵ Unfortunately, this conversion step is often incomplete when applied to higher oligosaccharides (e.g., hexamers) leading to inseparable mixtures and, in the presence of uronic acids, often leads to elimination products. Also, removal of tin byproducts can be extremely challenging. Phthalimido groups can pose challenges in base-catalyzed reactions,⁶ while an Alloc group prohibits the use of *N*-iodosuccinimide and related halonium ion-mediated activation strategies of thioglycosides.

In light of these limitations, fluorous protecting group versions that provide the added benefit of using the masking group for purification are of particular interest. To date, the synthesis and use of a fluorous alkyl carbamate-type (Troc-type) nitrogen-protecting group has been described for the protection and purification of amino sugars.^{7a} However, this protecting group synthesis involves the use of toxic chemicals such as mercury acetate and phosgene, thereby limiting its practical use to small scales.

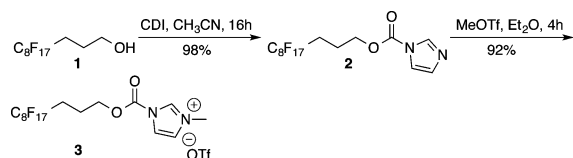
Our first goal then was to design a new carbamate-type amine-protecting group that could be made from nontoxic reagents amenable to scale up. A fluorous alkyl carbamate-type protecting group was chosen because alkyl carbamates not only can be deprotected using mild conditions like methyltrichlorosilane^{8a} or trimethylsilyl iodide^{8b} but also are known to survive most protection/deprotection conditions in carbohydrate synthesis as well as to provide stereoselectivity during glycosylations through neighboring group participation when placed next to the anomeric center.

Received: January 3, 2014

Published: February 10, 2014

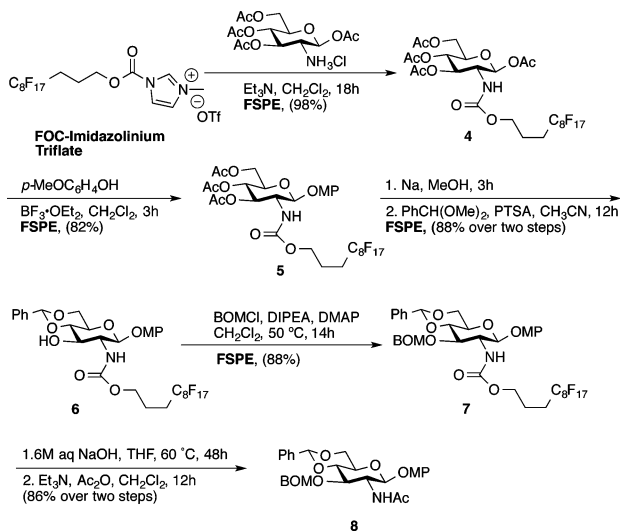
Imizadolinium carbamates are known for their use in the protection of amines.⁹ We hypothesized that attaching a fluororous chain five bonds distant from the reactive center would not alter the reactivity of this type of carbamate, and therefore, a new fluororous version of this protecting group could be produced. To this end, a fluororous protecting group **2**, with imidazole as a leaving group, was synthesized in one step starting from commercially available perfluorooctylpropanol **1** (Scheme 1). Unfortunately, reagent **2** was found to efficiently protect primary amino alcohols, but not glucosamine, even at elevated temperatures.

Scheme 1. Synthesis of FOC-imidazolium Triflate **3**



We envisioned that due to the relatively lower nucleophilicity and steric hindrance of the secondary amine group in glucosamine compared to the primary amino alcohol, a better leaving group than imidazole was needed in compound **2**. Fortunately, the addition of a reaction step to our initial design to produce such an improved leaving group was offset by the fact that, unlike previously reported versions of fluororous protecting groups for amines,⁷ the preparation of this new reagent did not require any chromatographic separation steps. The resulting reagent **3** (FOC, 1-(((4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptafluoroundecyl)oxy)carbonyl)-3-methyl-1*H*-imidazol-3-ium trifluoromethanesulfonate) was found to be stable at $-20\text{ }^{\circ}\text{C}$ for months under an argon atmosphere. However, the FOC reagent **3** could be easily added to glucosamine, and the resulting urethane was found to survive several common protection/deprotection conditions used in oligosaccharide and other small molecule synthesis, including basic and acidic conditions, as shown in Scheme 2. The group withstood a Lewis acid mediated glycosylation reaction, standard ambient temperature Zemplén deacylation conditions,

Scheme 2. Stability of the FOC Protecting Group under Various Conditions Common to Oligosaccharide Synthesis

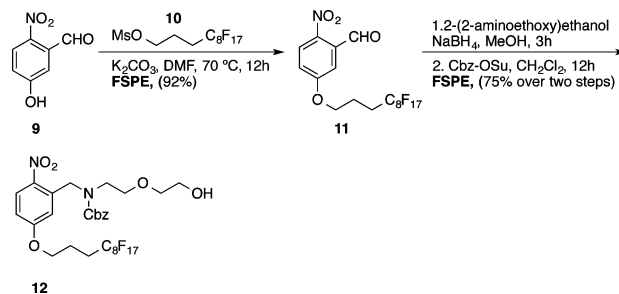


and protic acid mediated benzylidene acetal formation to eventually provide compound **6**.¹⁰ The free 3-OH was then protected by a benzylchloromethyl ether. Compounds **5–7** were all readily purified by FSPE using the fluorophilic solvent (methanol) to successfully elute the fluororous-tagged compounds from the C_8F_{17} -modified silica gel.

Because the new protecting group showed good stability under both acidic and basic conditions, work next turned to finding suitable conditions to remove the group. Several mild conditions known to remove alkyl carbamates were initially attempted (using trimethylsilyl iodide and methyl trichlorosilane respectively) to remove the FOC,⁸ but none were successful in removing the carbamate. After witnessing the stability of FOC under Zemplén conditions, we realized a stronger basic medium was needed for a nucleophile-mediated deprotection. Eventually, the free amine was generated using 6 M aqueous sodium hydroxide under reflux, which was subsequently acetylated to provide compound **8** (Scheme 2).

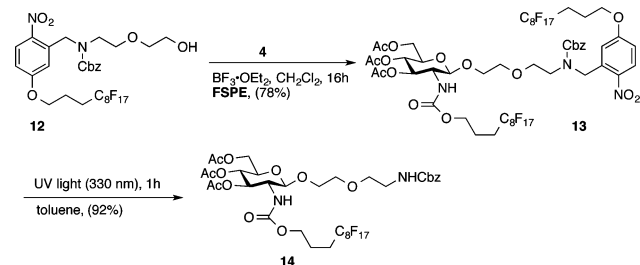
Our next objective was to design a fluororous amine-protecting group amenable to the synthesis of amine-terminated oligosaccharides, commonly used for attachment of these structures to arrays, and our first choice was a photolabile group, owing to its convenient deprotection conditions. Although *o*-nitrobenzyl carbamate groups are frequently used for photolysis,¹¹ a fluororous version of this type of protecting group amenable to oligosaccharide synthesis is still unknown. The fluororous component of the acceptor will enable us to synthesize various amine-terminated glycans in our automated solution-phase synthesis platform, where FSPE is used for purification of intermediates. Since upon photolysis the generated free primary amine could recombine with the *o*-nitrosoaldehyde side product,¹² we chose to refrain from making an *o*-nitrobenzyl carbamate group. Instead we decided to synthesize a fluororous *o*-nitrobenzylamine with an additional Cbz (benzyl chloroformate) group on the amine to prevent the lone pair of nitrogen from imine formation. With our design, once the desired chain length is obtained, oligosaccharides with an amine functional group at the reducing end protected with a Cbz group could be made using UV light; the Cbz group would then be removed during standard Pd-catalyzed hydrogenolysis of the benzyl ethers.¹³ A fluororous protecting group FNB (fluororous *o*-nitrobenzaldehyde) was then synthesized in one step from 5-hydroxy-2-nitrobenzaldehyde that can be used to mask amines through reductive amination. An acceptor **12** (Scheme 3) was made to probe the scope of photolysis of FNB in the presence of the FOC group. Compound **13** containing both protecting groups was synthesized from previously synthesized **4** and **12** and it was purified using gradation FSPE.^{3a} As expected, given that related groups are known to be

Scheme 3. Synthesis of FNB and a FNB-Protected Acceptor That Can Be Unmasked with Light



cleaved by light sources of wavelengths ranging from 300 to 365 nm,¹⁴ the Cbz-protected amine could be generated using UV radiation at 330 nm to provide amine **14** (Scheme 4) in

Scheme 4. Photodeprotection of FNB in the Presence of FOC



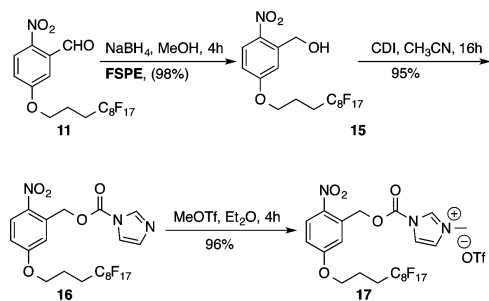
high yields while keeping the FOC protecting group intact. Even without the FOC group, the FNB-linker can render a molecule amenable to purification by FSPE (Scheme S7, Supporting Information).

Since more complex sugars such as heparin contain differentially modified amines, we next set out to test a fluorous protecting group for amino sugars orthogonal to FOC that could be removed by photolysis. Given the precedent of *o*-nitrobenzyl carbamates, which are known to generate free amines under UV light in a traceless manner,¹¹ we set out to test the feasibility of making a fluorous variant of this group, which we named FNBC (fluorous *o*-nitrobenzyl carbamate). In addition, a carbamate group was chosen in order to aid in forming stereoselective glycosylation products through anchimeric assistance.

Although removal of the *o*-nitrobenzyl carbamates is known to be a lower yielding reaction due to imine formation with the *o*-nitrosobenzaldehyde side product produced,^{11d} we decided to use a primary amine in the reaction mixture. We hypothesized that owing to the relatively low nucleophilicity and steric hindrance of an amine group in aminosugars, imine formation would be slower than with primary amines. This, in turn, could make the deprotection step higher yielding.^{11d}

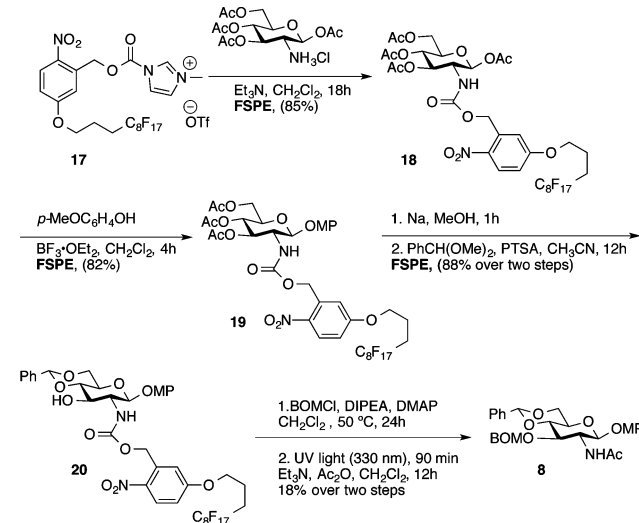
Ideally, a scheme to produce such a protecting group would also require minimal or no chromatographic separation steps. As before, we chose to have a methylimidazolium leaving group on the protecting group. Starting with a nucleophilic substitution reaction, followed by a sodium borohydride-mediated reduction, a nucleophilic addition–elimination reaction with carbonyl diimidazole, and formation of a methylimidazolium salt, we successfully synthesized FNBC **17** (Scheme 5).

Scheme 5. Synthesis of FNBC-imidazolium Triflate



We next tested the stability of this new protecting group under common reaction conditions used for oligosaccharide synthesis (Scheme 6). Starting from the peracetylated glucos-

Scheme 6. Stability of FNBC under Various Conditions Common to Oligosaccharide Synthesis



amine hydrochloride, we carried out carbamate formation, glycosylation, deacetylation, benzylidene acetal formation, and ether formation reactions; the protecting group was found to be stable to all these conditions. Unfortunately, this deprotection step proved to be as low yielding as prior reports of such light-mediated cleavage reactions on nonfluorous compounds with the formation of insoluble materials; using a primary amine as a competing nucleophile in the reaction did not improve the outcome.^{11d} Resubjecting the desired product **8** to the deprotection conditions resulted in complete reisolation of the material, thereby demonstrating that the final product is stable under the reaction conditions. The loss of material appears to be the result of the formation of insoluble byproducts. To probe whether the complex carbohydrate substrate was contributing to the low yields, an additional simpler FNBC-protected amino alcohol was synthesized (Scheme S8, Supporting Information). In this case also, the deprotection turned out to be low yielding with formation of insoluble byproducts. Realistically, this parent FNBC group, like its nonfluorous analogues, should be limited to the protection of secondary deactivated amines rather than primary amines. Given literature precedents,¹⁵ a second-generation FNBC-type protecting group better suited for primary amines could be envisioned with a methyl substitution at the benzylic position. This added methyl group should render the molecule more effective in the deprotection reaction owing to the low electrophilicity of the generated *o*-nitrosoacetophenone product.

In summary, three new fluorous protecting groups for amine functional groups have been designed and synthesized. These new masking groups should find use not only in oligosaccharide synthesis but also in the design of small molecules and in peptide synthesis where amine functional groups are also prevalent. Although the use of FNBC as a protecting group for aminosugars is limited owing to low yields during deprotections, an efficient photolabile amine-protecting group FNB has been developed to aid in the construction of linkers for

carbohydrates or other molecules. FNB was found to be orthogonal to FOC, and the deprotection step was found to be high yielding unlike that of FNBC. Work is ongoing to apply FNB in the synthesis of a library of *N*-glycans.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis protocol and characterization of all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: npohl@indiana.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

N.P. acknowledges the Joan and Marvin Carmack fund and Indiana University. This work was also supported in part by the National Institutes of Health (U19 A1091031).

■ REFERENCES

- (1) (a) Seeberger, P. H. *Chem. Soc. Rev.* **2008**, *37*, 19. (b) Ratner, D. M.; Adams, E. W.; Disney, M. D.; Seeberger, P. H. *ChemBioChem* **2004**, *5*, 1375.
- (2) (a) Curran, D. P.; Luo, Z. *J. Am. Chem. Soc.* **1999**, *121*, 9069. (b) Zhang, W. *Chem. Rev.* **2009**, *109*, 749. (c) Zhang, W. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 784. (d) Roychoudhury, R.; Pohl, N. L. B. Light fluororous tag assisted oligosaccharide synthesis. In *Modern Synthetic Methods in Carbohydrate Chemistry-From Monosaccharides to Complex Glycoconjugates*; Werz, D. B., Vidal, S., Eds.; Wiley-VCH, 2013; p 221. (e) Zhang, W.; Curran, D. P. *Tetrahedron* **2006**, *62*, 11837.
- (3) (a) Park, G.; Ko, K.-S.; Zakharova, A.; Pohl, N. L. *J. Fluorine Chem.* **2008**, *129*, 978. (b) Zhang, F.; Zhang, W.; Zhang, Y.; Curran, D. P.; Liu, G. *J. Org. Chem.* **2009**, *74*, 2594. (c) Manzoni, L. *Chem. Commun.* **2003**, *23*, 2930. (d) Kojima, M.; Nakamura, Y.; Takeuchi, S. *Tetrahedron Lett.* **2007**, *48*, 4431.
- (4) (a) Wang, Z.; Chinoy, Z. S.; Ambre, S. G.; Peng, W.; McBride, R.; de Vries, R. P.; Glushka, J.; Paulson, J. C.; Boons, G.-J. *Science* **2013**, *341*, 379. (b) Davis, B. G. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2137. (c) Mitchell, S. A.; Pratt, M. R.; Hruby, V. J.; Polt, R. *J. Org. Chem.* **2001**, *61*, 2327. (d) Mong, K. T.; Wong, C. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 4087. (e) Oberli, M. A.; Bindschadler, P.; Werz, D. B.; Seeberger, P. H. *Org. Lett.* **2008**, *10*, 905. (f) Love, K. R.; Seeberger, P. H. *J. Org. Chem.* **2005**, *70*, 3168.
- (5) Pauls, H. W.; Fraser-Reid, B. *Carbohydr. Res.* **1986**, *150*, 111.
- (6) (a) Sasaki, T.; Minamoto, K.; Itoh, H. *J. Org. Chem.* **1978**, *43*, 2320. (b) Costello, C. A.; Kreuzman, A. J.; Zmijewski, M. J. *Tetrahedron Lett.* **1996**, *37*, 7469.
- (7) (a) Manzoni, L.; Castelli, R. *Org. Lett.* **2006**, *8*, 955. (b) Zong, C.; Venot, A.; Dhamale, O.; Boons, G.-J. *Org. Lett.* **2013**, *15*, 342. (c) Luo, Z.; Williams, J.; Read, R. W.; Curran, D. P. *J. Org. Chem.* **2001**, *66*, 4261. (d) Filippov, D. V.; van Zoelen, D. J.; Oldfield, S. P.; van der Marel, G. A.; Overkleeft, H. S.; Drijfhout, J. W.; van Boom, J. H. *Tetrahedron Lett.* **2002**, *43*, 7809. (e) Matsugi, M.; Yamanaka, K.; Inomata, I.; Takekoshi, N.; Hasegawa, M.; Curran, D. P. *QSAR Comb. Sci.* **2006**, *25*, 713. (f) de Visser, P. C.; van Helden, M.; Filippov, D. V.; van der Marel, G. A.; Drijfhout, J. W.; van Boom, J. H.; Noort, D.; Overkleeft, H. S. *Tetrahedron Lett.* **2003**, *44*, 9013.
- (8) (a) Lott, R. S.; Chauhan, V. S.; Stammer, C. H. *J. Chem. Soc., Chem. Commun.* **1979**, 495. (b) Raucher, S.; Bray, B. L.; Lawrence, R. F. *J. Am. Chem. Soc.* **1987**, *109*, 442. (c) Yeung, B. K. S.; Adamski-

Werner, S. L.; Bernard, J. B.; Poulenat, G.; Petillo, P. A. *Org. Lett.* **2000**, *2*, 3135.

(9) Upadhyay, S.; Sharma, A.; Sharma, A.; Rajagopal, V.; Datla, P.; Bellary, A.; Prasad, S. S. *PCT Int. Appl.* 2009047792, 2009.

(10) (a) Grzyb, J. A.; Shen, M.; Yoshina-Ishii, C.; Chi, W.; Stanley, R. B.; Batey, R. A. *Tetrahedron* **2005**, *61*, 7153. (b) Heller, S. T.; Sarpong, R. *Tetrahedron* **2011**, *67*, 8851. (c) Heller, S. T.; Sarpong, R. *Org. Lett.* **2010**, *12*, 4572.

(11) (a) Prompinit, P.; Achalkumar, A. S.; Han, X.; Bushby, R. J.; Walti, C.; Evans, S. D. *J. Phys. Chem. C* **2009**, *113*, 21642. (b) Cameron, J. F.; Frechet, J. M. J. *J. Am. Chem. Soc.* **1991**, *113*, 4303. (c) Gautier, A.; Nguyen, D. P.; Lusic, H.; An, W.; Deiters, A.; Chin, J. W. *J. Am. Chem. Soc.* **2010**, *132*, 4086. (d) Amit, B.; Zehavi, U.; Patchornik, A. *J. Org. Chem.* **1974**, *39*, 192.

(12) (a) Ajayaghosh, A.; Pillai, V. N. R. *Tetrahedron* **1988**, *44*, 6661. (b) Chandra, B.; Subramaniam, R.; Mallik, S.; Srivastava, D. K. *Org. Biomol. Chem.* **2006**, *4*, 1730.

(13) (a) Bergmann, M.; Zervas, L. *Ber.* **1932**, *65*, 1192. (b) Sakaitani, M.; Hori, K.; Ohfuné, Y. *Tetrahedron Lett.* **1988**, *29*, 2983.

(14) Bochet, C. G. *J. Chem. Soc., Perkin Trans. 1* **2002**, 125.

(15) (a) Wu, J.; Tang, X. *Bioorg. Med. Chem.* **2013**, *21*, 6205–6211. (b) Klinger, D.; Landfester, K. *Macromolecules* **2011**, *44*, 9758–9772.