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Protecting-Group-Based Colorimetric Monitoring of Fluorous-Phase and Solid-Phase Synthesis of Oligoglucosamines

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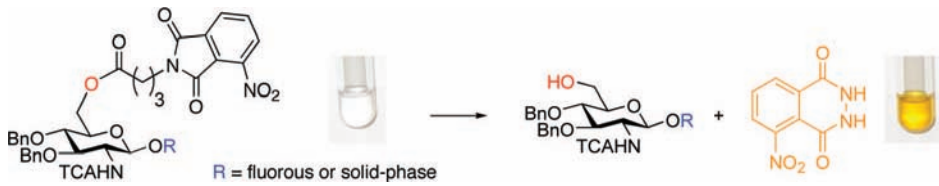
ABSTRACT



A new hydroxyl protecting group, nitrophthalimidobutyric (NPB) acid, has been synthesized in one solvent-free step for colorimetric monitoring of reaction cycles upon its facile removal with hydrazine acetate in the solid-phase and fluorous-phase syntheses of antigenic oligoglucosamines associated with infectious *Staphylococcus aureus*. The NPB group serves as a convenient hydroxyl protecting group that is stable to the basic conditions required for the synthesis of the common trichloroacetimidate protecting groups, the strongly acidic conditions used in glycosylation reactions, as well as conditions commonly used to remove silicon-based protecting groups.

To match the achievements of commercial automated solid-phase synthesis of other biopolymers such as nucleic acids and peptides, many scientists have tried to develop simple methods for the iterative synthesis of oligosaccharides. The first report of the solid-phase synthesis of oligosaccharides was described in 1971 with the production of a di- and trisaccharide.[1] Solid-phase synthesis can provide high yields by use of excess donors and has the advantages of ease of purification and synthesis automation when compared to conventional solution-phase methods. More recently, solid-phase synthesis of oligosaccharides has been automated by conversion of a standard peptide-synthesizer.[2] However, there are still unresolved complications in solid-phase synthesis of oligosaccharides that prevent its widespread use. A way to overcome the requirement for large excesses of donor groups is needed to make this practical. Furthermore, a method for monitoring the reaction progress on solid phase has been difficult to demonstrate because of trouble characterizing the product under homogeneous reaction conditions.[3] Methods to monitor such solid-phase processes include on-resin color tests,[4] high-resolution magic angle spin NMR,[5] gated decoupling NMR spectroscopy using ^{13}C-enriched tags,[3,6] ^{19}F NMR spectroscopy,[7] and MALDI-TOF

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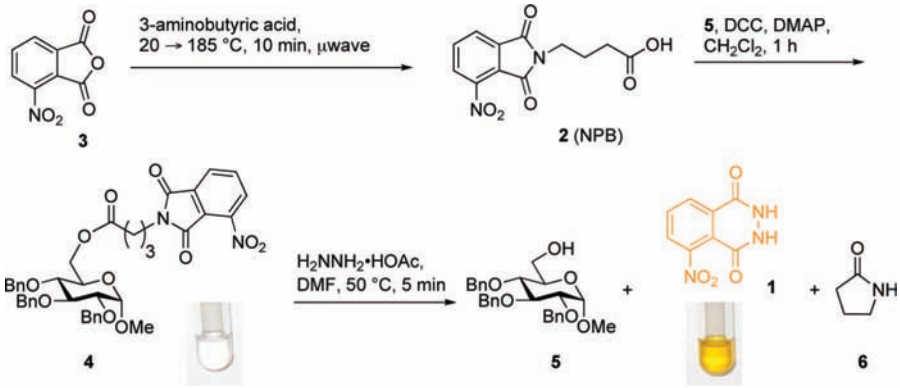
Scheme 1. Design of Colorimetric Protecting Group for Hydroxyl-Group Protection[a]



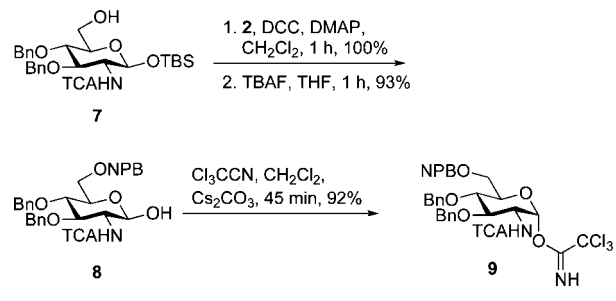
[a] Synthesis of the nitrophthalimidobutyric (NPB) acid can be used as protecting group to mask a carbohydrate hydroxyl group. The cleavage reaction resulted in an orange-colored solution.

mass spectroscopy.[8] However, these methods are difficult to incorporate readily into automated processes. Commercial peptide synthesizers often rely on colorimetric monitoring of deprotection cycles using fluorenylmethoxycarbonyl (Fmoc) that thereby allows continuous feedback during the synthesis.[9] The efficiency of a coupling cycle can be inferred from the amount, monitored by UV−vis spectroscopy, of the released masking group prior to the next coupling cycle.

The use of Fmoc in the solid-phase synthesis of carbohydrates has been shown.[10] However, the extreme base sensitivity of Fmoc groups render their introduction into building blocks challenging. In addition, unlike peptides and nucleic acids, carbohydrates often include branched sequences. Therefore, a second less base-sensitive protecting group that produces a color signature upon removal is needed. Herein we report the design and synthesis of a new hydroxyl protecting group for colorimetric monitoring of reaction cycles and report its use in the solid-phase and fluorous-phase synthesis of antigenic oligoglucosamines[11] associated with infectious *Staphylococcus aureus*. The alternative fluorous tag-assisted solution-phase strategy[12] has the advantage of requiring significantly fewer equivalents of donor building blocks than the solid-phase approach.

The design of colored groups led us first to an investigation of dye molecules. Unfortunately, many of these compounds have inconvenient functional groups such as sulfates that would likely complicate the glycosylation reactions. The observation that nitrophthalhydrazide (**1**), a common intermediate in the undergraduate laboratory synthesis of luminol,[13] was a colored solid led us to think of strategies to generate this compound in the process of deprotecting a hydroxyl group. Retrosynthetic analysis pointed to opening of a nitrophthalic anhydride or imide with hydrazine. This reasoning led us to our final target molecule: nitrophthalimidobutyric (NPB) acid (**2**). The synthesis of NPB was readily accomplished in 92% or greater yield by melting of 4-nitrophthalic anhydride (**3**) with inexpensive 3-aminobutyric acid or, more easily, by microwave irradiation of the neat compounds (Scheme 1). The group can be added to a

Scheme 2. Synthesis of NPB-Protected Glucosamine Building Block



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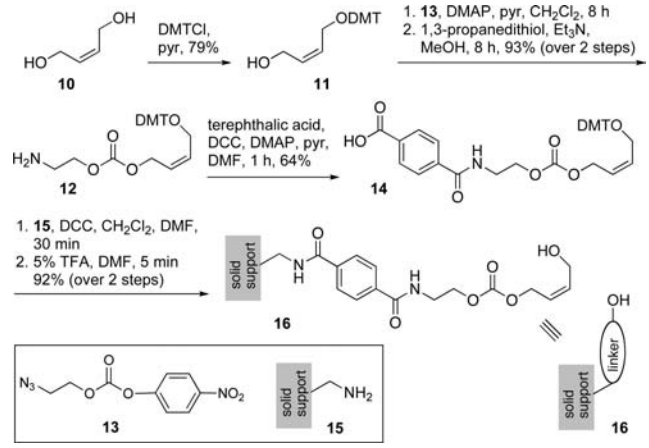
hydroxyl using standard carbodiimide[14] coupling conditions. Subsequent deprotection of NPB went smoothly using buffered hydrazine acetate in DMF at 50 °C to provide 3-nitrophthalhydrazide (**1**), which has an orange color in solution as well as byproduct **6** at comparable rates. Using Beer's law, we calculated the molar extinction coefficient (ε) of 3-nitrophthalhydrazide at 432 nm to be 4.8 M^{-1} cm^{-1} in DMF.

The next step was to test this strategy on solid phase. We decided to use solid-phase oligosaccharide synthesis using NPB as a temporary protecting group for the synthesis of 1,6-linked bacterial oligoglucosamines found on the surface of *S. aureus* associated with human infections. This target has been made before by automated solid-phase synthesis, but without any type of reaction monitoring.[15] To this end, we commenced the synthesis of glycosyl donor **9** (Scheme 2). The NPB group was installed on the known primary alcohol **7**[15] in high yield. After the silyl group was cleaved with tetrabutylammonium fluoride (TBAF) to reveal alcohol **8**, a trichloroacetimdate was readily installed with trichloroacetonitrile and Cs_2CO_3 as a base to yield glycosyl donor **9**.

We next required a linker to attach to a solid phase. Because of ease of handling compared to conventional powdered polystyrene resins, we chose a solid support grafted with polystyrene[16] modified with a reactive aminomethyl group. The synthesis of the linker, after much trial and error, commenced with monoaddition of a DMT group to *cis*-1,4-butenediol **10** (Scheme 3). The resulting free alcohol was reacted with azide **13**, which was subsequently converted to an amine by treatment with 1,3-propanedithiol and triethylamine. Coupling of the amine with terephthalic acid provided linker **14**. The linker was coupled to the aminomethylated resin via an amide; deprotection of the trityl protecting group under acidic conditions in DMF revealed solid-supported alcohol **16** in 91% yield from acid **14**. This alcohol was then coupled to glycosyl donor **9** using trimethylsilyltriflate in dichloromethane; the coupling reaction was repeated (Scheme 4). Reaction yields were calculated upon removal of the NPB group in hydrazine acetate and confirmed by weighing the resin after thorough rinsing and solvent removal. Yields for the coupling (2×)/deprotection cycle were 98% at the monomer stage and 96% at the dimer stage but unfortunately required a large excess of the glycosyl donor to produce the dimer.

To avoid such large excesses of donor usage, we next tried a fluorous tag-assisted strategy that should allow the rapid

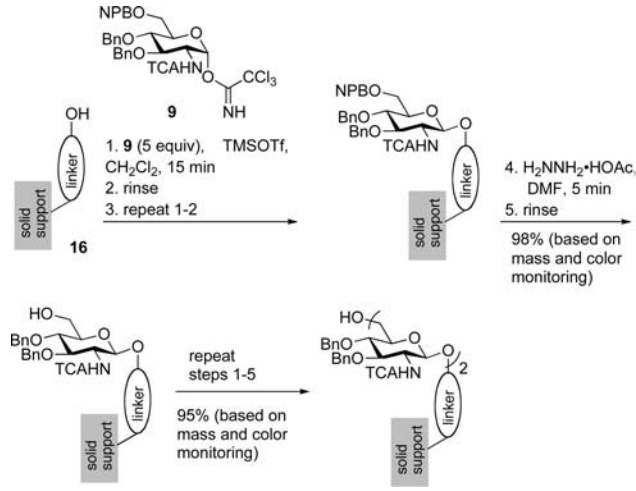
Scheme 3. Synthesis of Linker for SynPhase Lantern Support



solution-phase synthesis of oligosaccharides in a manner also amenable to automation (Scheme 5). This approach has the added benefit of allowing direct incorporation of fluorous-tagged sugars into a microarray platform for biological screening.[17,18] To this end, the fluorous-tagged allyl linker **17**[18] was coupled to donor **9**. The coupling and deprotection cycles were repeated as for the solid-phase synthesis with analysis of the colored deprotection byproduct to track yields. Since the coupling reactions were performed in solution, they required only 1.5 equiv of the sugar donor, and double coupling was unnecessary to provide the fluorous-tagged mono-, di-, and trisaccharides **18**−**20** in high yields using fluorous solid-phase extractions[19] in place of resin rinsing. Clearly, a solution-phase approach allows significant building block savings. Although obviously the solution-based fluorous-tag approach also leaves open other traditional monitoring techniques, the NPB group proved valuable in monitoring both solid-phase and solution-based syntheses.

In conclusion, the NPB group serves as a convenient hydroxyl protecting group that is stable to the basic condi-

Scheme 4. Synthesis of Glucosamine Dimer on a Solid Support



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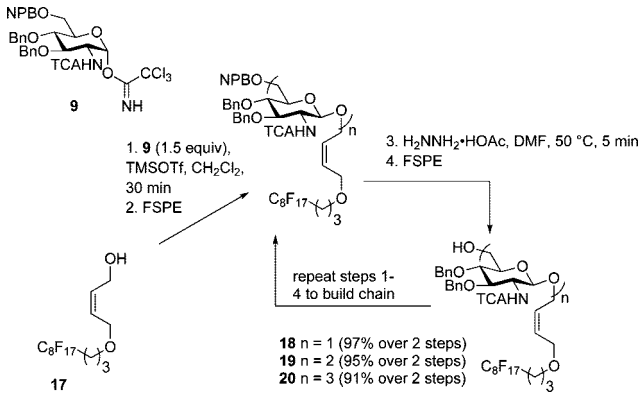
Scheme 5. Synthesis of Glucosamine Oligomers on a Fluorous Support Using Fluorous Solid-Phase Extractions (FSPE)



tions required for the synthesis of the common trichloroacetimidate protecting groups, to the strongly acidic conditions used in glycosylation reactions, and to conditions commonly used to remove silicon-based protecting groups. Treatment with buffered hydrazine, however, readily removes the group with production of an orange-colored byproduct that allows reaction monitoring by UV−vis. The NPB group could be used in the synthesis of protected solid-phase-bound and fluorous-tagged 1,6-linked β-glucosamine oligomers, but the synthesis was much more efficient when performed on the fluorous support. Ultimately, the fluorous tag-assisted solution-phase strategy, in conjunction with colorimetric or other more traditional monitoring techniques, should prove feasible for the rapid and potentially automated modular synthesis of carbohydrates and their use in forming microarrays for bioassays.

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Supporting Information Available: Experimental procedures and copies of ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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