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Froc: A New Fluorous Protective Group for Peptide and Oligosaccharide Synthesis†

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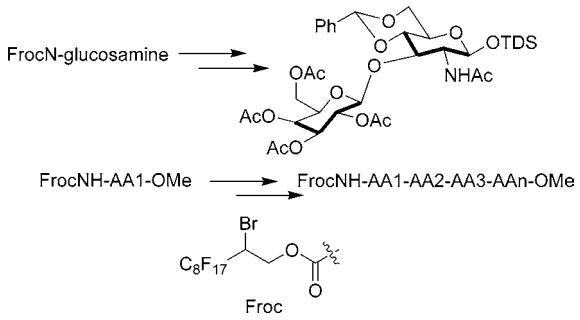
ABSTRACT



The synthesis of a new fluorous protecting group, Froc, is described. This new fluorous tag has been used in peptide and carbohydrate synthesis by our group and readily allows us to fully characterize each product (NMR, MS) and monitor each synthetic step by TLC. Purification of the products is generally performed by standard fluorous solid-phase extraction techniques (e.g., F-SPE), but standard chromatographic purifications are also possible if required.

The use of fluorous techniques[1] for the separation of reaction mixtures has found wide attraction in synthetic organic disciplines in recent years.[2] Fluorous chemistry has been studied in several fields such as catalytic chemistry, combinatorial chemistry, parallel synthesis,[3] and very recently in carbohydrate microarrays.[4] An important methodology is the so-called "light-fluorous" strategy developed by Curran and co-workers,[1a,5] in which fluorous compounds (40% or less fluorine content by MW) are readily separated from nonfluorinated compounds by a simple extraction (F-SPE). For these reasons, an array of fluorinated protecting group have become available in recent years,[6] including fluorous versions of the Z,[7] Boc,[8] *t*-Bu,[9] Bn,[10] THP,[11] Msc,[12] acyl-based,[13] silyl-based,[14] alkoxyethyl ether,[15] and alcohol[16] protecting groups. Peptides and carbohydrates can be easily prepared by solid-phase syntheses that allow a very simple

† Dedicated to Professor Carlo Scolastico on the occasion of his 70th birthday.

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purification by filtration. However, the method suffers from some serious disadvantages, such as reduced reactivity and the inability to monitor the coupling reactions by TLC, NMR, and mass spectrometry.

We reasoned that a reducible fluorous protecting group would be an attractive alternative tag for standard SPPS (solid-phase peptide synthesis) and solid-phase carbohydrate synthesis. Trichloethoxycarbonyl (Troc) is frequently used in organic synthesis,[17] especially where amino sugar derivatives[18] are involved. This is due to its stability under mild acidic and basic conditions and its ease of removal under specific conditions.[18a−c,19] Accordingly, we set out to design, synthesize and apply a fluorous version of the trichloethoxycarbonyl (Figure 1) protecting group for amines. The new

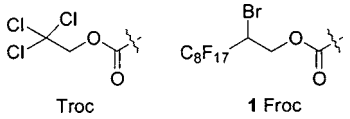
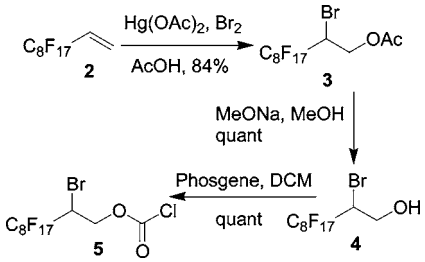


Figure 1. Troc protecting group and the Froc tag **1**.

fluorous protecting group **1** was named Froc by analogy with its nonfluorous Troc counterpart. The synthetic route for the preparation of the fluorous tag **1** is shown in Scheme 1.

Scheme 1. Synthesis of the FrocCl

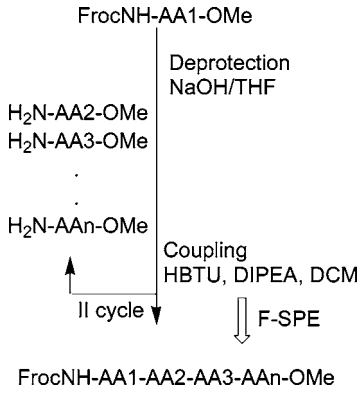


Alcohol **4** is obtained via a small modification of a published protocol.[20] In the first step, commercially available 1*H*,1*H*,2*H*,2*H*-perfluoro-1-decene **2** is treated with $Hg(OAc)_2$ in the presence of Br_2 in AcOH to give **3**. The target compound **5** is thereafter obtained in an excellent overall yield (84%) by chloroformylation of **4**.[21]

With the fluorous tag in hand, introduction and removal of the Froc protecting group was explored.

To demonstrate the versatility and utility of the Froc protecting group in peptide synthesis, we prepared the Gly-Gly dipeptide and the bioactive peptide RGD. The peptides were prepared following the general procedure shown in Scheme 2. The amino acids were commercial or easily

Scheme 2. General Scheme for the Peptide Synthesis Using Froc as Amino Protecting Group



prepared from a commercial source. The first amino acid was protected at the amino group with the freshly prepared FrocCl to give the Froc-protected amino acid. As a proof of concept, treatment of FrocHN-Gly-OMe with $Zn/Ac_2O/Et_3N$ quantitatively gave the AcHN-Gly-OMe derivative. With this result in hand, we started the peptide synthesis. After every step, it was possible to characterize all the intermediates, and if starting materials were revealed, a second cycle of reaction could be performed to drive the reaction to completion. Just like in solid-phase peptide synthesis, where an

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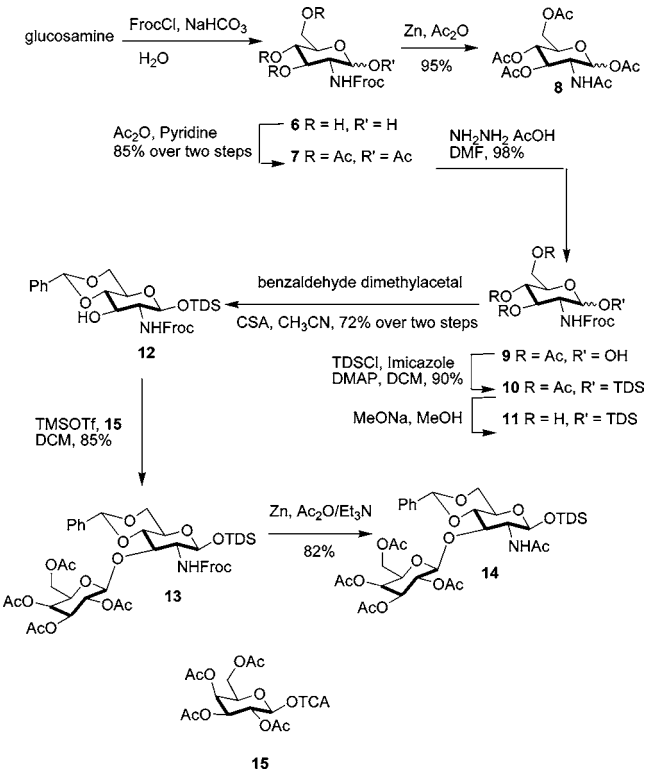
(21) **5** was reacted with $BnNH_2$ to give the benzyl carbamate.

excess of coupling reagent can be used, the excess reagent can subsequently removed by a simple F-SPE. For the coupling of the amino acids we decided to use a standard protocol normally applied in SPPS (HBTU, DIPEA, DCM).

With the aim of using the fluorous technology to speed up the synthesis of oligosaccharides,[22] we decided to use glucosamine as a model compound. Glucosamine was reacted with FrocCl **5** to give the corresponding Froc-protected glucosamine **6**, which was peracetylated with Ac_2O in pyridine (Scheme 3). Treatment of **7** with Zn/Ac_2O quantitatively gave a one-pot conversion of the starting sugar into the *N*-acetylglucosamine derivative (Scheme 3).

Scheme 3. Synthesis of a Disaccharide Using Froc



On the basis of these results, we turned our attention to application of the Froc protecting group to oligosaccharide synthesis. The synthetic scheme examinated employs many of the reagents, protecting groups manipulations and glycosylation conditions that are standard in the field (Scheme 3). So, starting from the peracetylated *N*-Froc-protected *O*-acetylglucosamine **7**, the acetyl group in the anomeric position was removed by hydrazinium acetate to give **9** in 98% yield. The texyldimethylsilyl group was attached to the anomeric hydroxy group of compound **9** by using imidazole and DMAP to give compound **10** in 90% yield. Removal of the acetyl groups from **10** under Zemplén conditions[23] followed by treatment of the crude product with benzaldehyde dimethyl acetal in the presence of CSA afforded the fluorous glycosyl acceptor **11** (72% over two steps). The disaccharide Galβ1,3-GlcNAc **13** was obtained by reaction of the pure acceptor **11** with the tetraacetylated galactose trichloroacetimidate (as glycosyl donor) in the presence of TMSOTf in CH_2Cl_2 (85%). This synthetic scheme showed that the new protecting group is capable of withstanding the typical reaction conditions required for protection/deprotection of a glycosyl acceptor and for glycosylation reactions. Due to the limited number of fluorines in the tag, compounds **6**, **7**, and **9**−**13** maintain a normal chromatographic behavior on standard silica gel, and all compounds can be characterized by NMR and mass spectrometry. The reactions can also be readily monitored by TLC. Furthermore, all fluorous tag compounds can be separated from the reaction mixture by a simple solid-phase extraction (F-SPE); the nonfluorinated compounds can be eluted with 80% MeOH/H_2O, and the fluorous compounds can be recovered from the column with MeOH. A second reaction cycle can be performed if some starting material is observed (TLC, MS, and NMR). The new protecting group can be easily removed and substituted with an acetyl group by treatment with $Zn/Ac_2O/Et_3N$ to give **14** in 82% yield.

In summary, we have developed and applied a new fluorous protecting group we have named Froc for its analogies with the nonfluorous trichloroethoxycarbonyl (Troc) counterpart. This new protecting group can be easily used for peptide and carbohydrate synthesis. All fluorinated compounds synthesized retain normal chromatographic behavior on standard silica gel, allowing easy monitoring of the course of reactions by TLC. Purification of the desired products obtained from multistep synthesis was significantly simplified by the fluorous moiety. The reaction mixtures were subjected to F-SPE on fluorous silica gel. The fluorous compounds could also be purified by normal silica gel chromatography if necessary, which was advantageous, compared to solid-phase synthesis. In addition, each synthetic intermediate could be easily characterized by NMR and mass spectroscopy (MALDI-TOF, ESI). The Froc group contains a stereogenic center but this have no effect on the interpretation of the spectra of chiral compounds. The spectra look reasonably clean and can be interpretated (see Supporting Information).

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

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