ORGANIC LETTERS

2000
Vol. 2, No. 20
3043−3046

O-Glycosyl Trichloroacetimidates Bearing Fmoc as Temporary Hydroxy Protecting Group: A New Access to Solid-Phase Oligosaccharide Synthesis

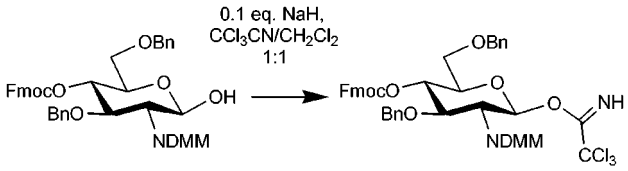
Fabien Roussel, Laurent Knerr,[†] Matthias Grathwohl, and Richard R. Schmidt*

Fakultät für Chemie, Universität Konstanz, Fach M 725, D-78457 Konstanz, Germany

richard.schmidt@uni-konstanz.de

Received May 19, 2000

ABSTRACT



Different *O*-glycosyl trichloroacetimidates bearing base sensitive Fmoc protected hydroxy groups were efficiently prepared with CCl_3CN using a catalytic amount of sodium hydride. The resulting glycosyl donors were engaged in glycosylation reactions both in solution and on solid support with a new ester-type linker with good results. In both approaches, Fmoc groups were afterward quantitatively cleaved using mild basic conditions.

The synthesis of complex oligosaccharides requires extremely elaborate protecting group strategies. Therefore the study of new hydroxy and amino protecting groups is of great interest in this field. Particularly, the synthesis of oligosaccharides on polymer supports[1] requires protecting groups that are cleaved under mild conditions and are compatible with the linker system. In the course of our work on the use of *O*-glycosyl trichloroacetimidates[2] for the synthesis of oligosaccharides on solid support, we became interested in the elaboration of a new temporary hydroxy protecting group that is cleaved under mild basic conditions. Indeed, acetyl is the most popular nucleophile labile temporary protecting group applied in solid-phase oligosaccharide synthesis. However, its cleavage on solid support requires rather strong alkaline conditions precluding the use of simple ester-type linker systems, which have already proven useful in the field.[3] Thus we decided to initiate a study of base labile hydroxy protecting groups that are compatible with ester-type linkers.

Among the possible protecting groups envisaged, the 9-fluorenylmethyloxycarbonyl (Fmoc) group appeared particularly attractive. Although very popular as an amino protecting group in peptide solid-phase synthesis, we were surprised to find only a few reports on its use as a hydroxy protecting group[4] in carbohydrate chemistry, whether in solution[5] or on solid phase.[6] Moreover, no application of Fmoc as hydroxy protecting group in oligosaccharide synthesis using *O*-glycosyl trichloroacetimidates[7] as glycosyl donors was reported. We describe here the syntheses of

[†] Present adress: UCB Pharma S.A., Chemin du Foriest 1420 Braine l'Alleud, Belgium.

(1) For a recent review see: Osborn, H. M. I.; Khan, T. H. *Tetrahedron* **1999**, *55*, 1807.

(2) (a) Knerr, L.; Schmidt, R. R. *Synlett* **1999**, 1802. (b) Knerr, L.; Schmidt, R. R. *Eur. J. Org. Chem.* **2000**, 2803. (c) Rademann, J.; Geyer, A.; Schmidt, R. R. *Angew. Chem.* **1998**, *110*, 1309; *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1241. (d) Rademann, J.; Schmidt, R. R. *J. Org. Chem.* **1997**, *62*, 3650. (e) Heckel, A.; Mross, E.; Jung, K. H.; Rademann, J.; Schmidt, R. R. *Synlett* **1998**, 171.

(3) (a) Shimizu, H.; Ito, Y.; Kanie, O.; Ogawa, T. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2841. (b) Manabe, S.; Ito, Y.; Ogawa, T. *Synlett* **1998**, 628.

(4) Gioeli, C.; Chattopadhyaya, J. B. *J. Chem. Soc., Chem Commun.* **1982**, 672.

(5) Freese, S. J.; Vann, W. F. *Carbohydr. Res.* **1996**, *281*, 313.

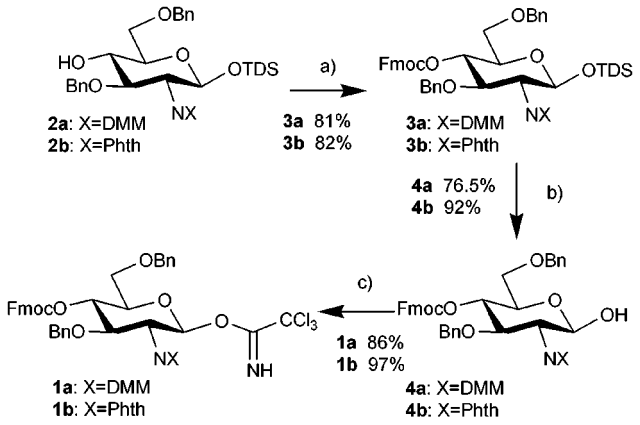
(6) (a) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; DeRoose, F. *J. Am. Chem. Soc.* **1997**, *119*, 449. (b) Nicolaou, K. C.; Watanabe, N.; Li, J.; Pastor, J.; Winssinger, N. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1559. (c) Zhu, T.; Boons, G.-J. *Tetrahedron: Asymmetry* **2000**, *11*, 199.

10.1021/ol006081l CCC: $19.00
Published on Web 09/06/2000

O-glycosyl trichloroacetimidates donors **1a**−**g** containing Fmoc protection and their use as glycosyl donors in solution and on solid support.

The main difficulty associated with the preparation of these *O*-glycosyl trichloroacetimidates was to find conditions compatible with the presence of this base sensitive group all along the synthesis. Most of all, the last step of introduction of the trichloroacetimidate moiety had to be performed in basic conditions[7b] and seemed to be problematic. However, the synthetic sequence described in Scheme 1 gave excellent results in all cases studied until now. For

Scheme 1[a]

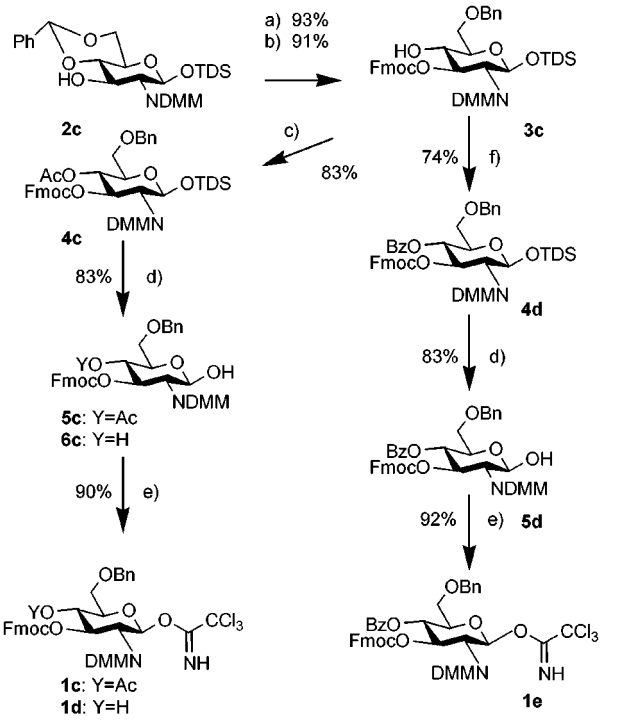


[a] (a) FmocCl, DMAP, pyridine. (b) Excess HF·pyridine, THF, rt. (c) 0.1 equiv NaH, (**1a**) CH_2Cl_2/CCl_3CN (1/1), (**1b**) CCl_3CN, rt.

all the building blocks prepared, we have chosen to use the thexyldimethylsilyl group (TDS) to protect the anomeric position. Thus the *O*-TDS-protected compounds (**2a**−**g**)[8] were prepared. In all cases, the Fmoc group was introduced using FmocCl with a catalytic amount of DMAP in pyridine at room temperature in very good yields. To optimize the desilylation step, *N*-DMM-protected[9] glucosamine derivative **3a** and the *N*-phthalimido derivative **3b** were chosen as models. The best results were obtained using an excess of HF·pyridine complex in THF at room temperature[10] (92%). Among the methods available to perform the formation of the trichloroacetimidate function,[7b] the use of a catalytic amount of NaH as the base seemed particularly attractive to avoid the cleavage of the Fmoc group via β elimination. Thus, in the presence of less than 0.1 equiv of NaH in trichloroacetonitrile, alcohol **4b** was converted into β-*O*-glycosyl trichloroacetimidate **1b**[11] in 97% yield after filtration on a small pad of silica.[12] The reaction proved to be very fast and clean. The same procedure applied to a similar building block, namely, *N*-DMM-protected **4a**, confirmed the efficiency of this method. β-*O*-Glycosyl trichloroacetimidates **1a**[11] and **1b** are of great interest for the construction of carbohydrates of the *lacto*-series of glycosphingolipids and of *N*-glycans.

These good results prompted us to investigate the compatibility of our methodology with other protective groups. At first, the synthesis of glucosamine derivatives **1c**[11] and **1e**[11] was investigated (Scheme 2). These compounds were

Scheme 2[a]



[a] (a) FmocCl, DMAP, pyridine. (b) $NaBH_3CN$, HCl·Et_2O, THF. (c) Ac_2O, pyridine. (d) Excess HF·pyridine, THF, rt. (e) 0.1 equiv NaH, CH_2Cl_2/CCl_3CN (1/1), rt. (f) 2.5 equiv BzCl, pyridine.

synthesized using **2c** as starting material. After linkage of the Fmoc group to the O3 position (93% yield), the benzylidene acetal function was regioselectively opened using $NaBH_3CN$ and a solution of HCl·Et_2O[13] to afford **3c**

(7) (a) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212. (b) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.

(8) (a) For **2a** and **2c** see: Chiesa, V.; Schmidt, R. R. *Eur. J. Org. Chem.*, accepted for publication. (b) For **2b** see: Grathwohl, M. Diplomarbeit, Universität Konstanz, Germany, 1997. (c) For **2f** see: Peter, J. Diplomarbeit, Universität Konstanz, Germany, 1994. (d) For **2g** see: Knerr, L.; Roussel, F.; Schmidt, R. R., unpublished results.

(9) Aly, M. R. E.; Castro-Palomino, J. C.; Ibrahim, E. I.; El-Ashry, E. H.; Schmidt, R. R. *Eur. J. Org. Chem.* **1998**, 2305.

(10) Trost, B. M.; Caldwell, C. G.; Murayama, E.; Heissler, D. *J. Org. Chem.* **1983**, *48*, 3252.

(11) NMR selected data for compounds **1a**−**g**: ^{1}H NMR (600 MHz, $CDCl_3$) δ **1a** 6.23 (d, $J_{1,2}$ = 8.8 Hz, 1-Hβ); **1b** 6.41 (d, $J_{1,2}$ = 8.4 Hz, 1-Hβ); **1c** 6.38 (d, $J_{1,2}$ = 8.7 Hz, 1-Hβ); **1d** 6.36 (d, $J_{1,2}$ = 8.7 Hz, 1-Hβ); **1e** 6.54 (d, $J_{1,2}$ = 8.9 Hz, 1-Hβ); **1f** 6.85 (d, $J_{1,2}$ = 3.4 Hz, 1-Hα); **1g** 6.40 (d, $J_{1,2}$ = 3.5 Hz, 1-Hα).

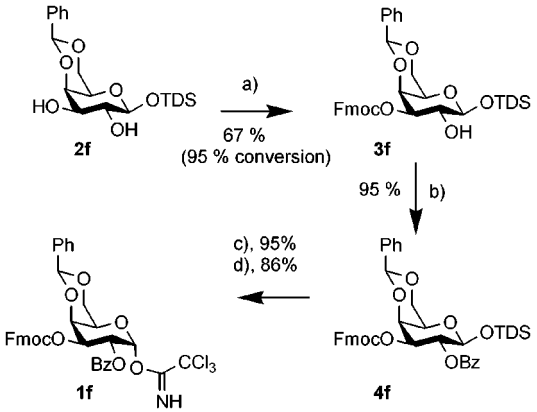
(12) **Typical Procedure**. Compound **4b** (115 mg, 0.16 mmol) was dissolved in CCl_3CN (3 mL) under Ar. Sodium hydride (0.1 equiv, 0.5 mg) was added to the solution, and after 10 min of stirring, a TLC analysis showed a complete consumption of the starting material. The solution was adsorbed on silica, and the solvents were evaporated in vacuo. The residue was purified by flash chromatography (very small amount of silica) to afford imidate **1b** in a 97% yield.

(13) Garegg, P. J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97.

in 91% yield. At first, we have introduced an acetyl group in the O4 position using acetic anhydride in pyridine to give compound **4c** in a yield of 83%. During the desilylation step with HF·pyridine, partial loss of the acetyl group was observed. Thus, an inseparable mixture of compounds **5c** and **6c** (61/39) was obtained in 83% yield. The imidate moiety was introduced in this mixture of sugars to afford *β*-*O*-glycosyl trichloroacetimidate **1c** and **1d**,[11] separable by flash chromatography, with 90% yield. It is worthwhile to note that no trace of trichloroacetimidate function at O4 position was observed. To improve the resistance of the protective group at O4 position to the acidic conditions of desilylation, a benzoyl group was attached instead of an acetyl group. At first, we tried to introduce this ester group on compound **3c** by reaction with benzoic anhydride in pyridine. Unfortunately, the major compound isolated had after the loss of the Fmoc group benzoyl groups at O3 and O4 positions. The transformation of **3c** into **4d** was optimized by using benzoyl chloride in pyridine (best conditions, 2.5 equiv BzCl, pyridine, 12 h) to afford compound **4d** with a yield of 74%. Only 13% of the dibenzoylated compound was obtained. After desilylation (**4d** → **5d**, 83% yield) and formation of the trichloroacetimidate function (92% yield) under the previous conditions, only glycosyl donor of *β* configuration **1e** was produced. Glycosyl donors **1c** and **1e** are very interesting for the synthesis of oligosaccharides of the *lactoneo*-glycosphingolipid family.

The same strategy was also applied to the galactosyl derivative **2f** bearing two hydroxy functions in C2 and C3 positions (Scheme 3). An interesting regioselective introduc-

Scheme 3[a]

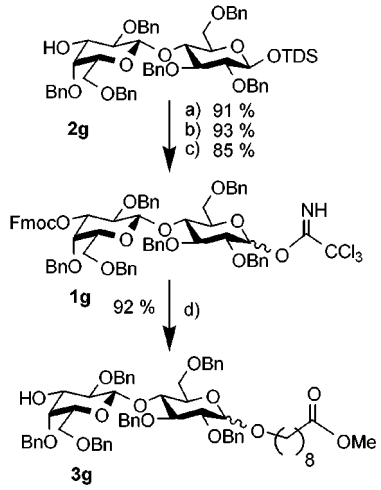


[a] (a) FmocCl, DMAP, pyridine/CH_3CN. (b) BzCl, CH_2Cl_2/pyridine (2/1). (c) Excess HF·pyridine, pyridine, 0 °C. (d) 0.1 equiv NaH, CH_2Cl_2/CCl_3CN (1/1), 0 °C.

tion of the Fmoc group in the more reactive O3 position of the galactoside was observed to afford **3f** in 67% yield (95% of conversion). A first attempt to install a benzoyl group at O2 position with benzoyl cyanide in acetonitrile with a catalytic amount of DMAP resulted in the loss of the Fmoc group. As previously, best conditions were the use of benzoyl chloride in a mixture of CH_2Cl_2/pyridine (2:1) to give compound **4f** in 95% yield. In this case, no loss of the Fmoc group was observed. The sensitivity of the benzylidene group to acidic conditions was circumvented by performing the desilylation in pyridine instead of THF at low temperature (95% yield). Treatment with trichloroacetonitrile according to the previous conditions gave α-*O*-galactosyl trichloroacetimidate **1f**[11] (containing only trace amounts of *β*-anomer) in 86% yield.

This general methodology was also applied to lactose derivative **2g** to afford in three steps an anomeric mixture (1:1) of trichloroacetimidate derivative **1g**[11] in 72% yield over three steps (Scheme 4). To show the usefulness of

Scheme 4. Glycosylation in Solution[a]



[a] (a) FmocCl, DMAP, pyridine. (b) Excess HF·pyridine, THF, rt. (c) 0.1 equiv NaH, CH_2Cl_2/CCl_3CN (1/1), rt. (d) $HO(CH_2)_8CO_2Me$, TMSOTf, CH_2Cl_2.

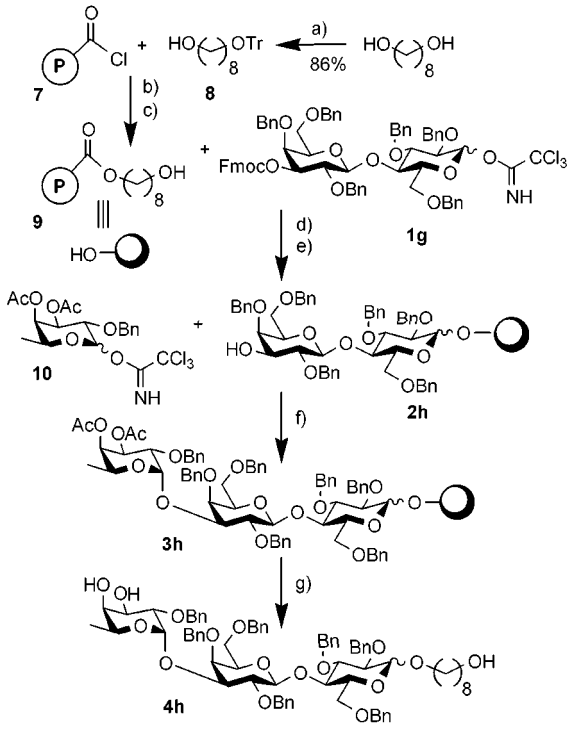
glycosyl donor **1g** as building block, it was employed in a glycosylation reaction. Thus, coupling with the Lemieux spacer[14] (methyl 9-hydroxynonanoate) in dichloromethane with TMSOTf as catalyst was carried out. In a "one-pot" procedure, triethylamine was added to afford after workup derivative **3g** as an anomeric mixture (1:1) in 92% yield.

Glycosyl donor **1g** was also employed in a glycosylation reaction on solid phase (Scheme 5). For this endeavor a new ester-type linker was selected. To this end acid chloride support **7**, derived from a commercial carboxypolystyrene resin (100−200 mesh, 1% cross-linked, 2 mmol/g)[15] was reacted with alcohol **8** (0.29 equiv) in pyridine at room temperature followed by capping with anhydrous methanol. Trityl groups were removed by treatment with trifluoroacetic acid allowing the calculation of the loading by colorimetric assay (0.53 mmol/g), thus affording linker-loaded resin **9**.

Reaction of **9** with lactosyl donor **1g** (3 equiv) upon activation with TMSOTf (0.3 equiv) in dichloromethane gave the corresponding glycosylated resin. This glycosylation step

(14) Lemieux, R. U.; Bundle, D. R.; Baker, D. A. *J. Am. Chem. Soc.* **1975**, *97*, 4076.
(15) Panek, S. P.; Zhu, B. *Tetrahedron Lett.* **1996**, *37*, 8151.

Scheme 5. Application on Solid Phase[a]



[a] (a) TrCl, DMAP, pyridine, rt. (b) pyridine, rt, 30 h then MeOH, 16 h. (c) 5% TFA, CH_2Cl_2/MeOH 9:1. (d) TMSOTf, CH_2Cl_2, rt. (e) CH_2Cl_2/NEt_3 (4/1), rt. (f) TMSOTf, CH_2Cl_2/dioxane 1:1, −25 °C. (g) MeONa, CH_2Cl_2/MeOH 9:1, rt.

was carried out twice to ensure a maximum functionalization. This step, as the next steps, were monitored by TLC and MALDI-TOF analysis of the cleavage product (MeONa, CH_2Cl_2/MeOH) from a small amount of resin (2 mg). The Fmoc group was easily removed by treatment with a dichloromethane/triethylamine mixture (4/1) at room temperature to afford resin **2h**. The disaccharide moiety was elongated with known fucosyl donor **10**[16] using the previously described experimental conditions[2d] to obtain α-anomeric linkage in resin **3h**. After analytical cleavage, not even trace amounts of the preceding disaccharide were observed. Cleavage from the solid support was carried out by using basic conditions (5 equiv MeONa, CH_2Cl_2/MeOH 9:1, 10 h, rt) to give, after purification by flash chromatography, an anomeric mixture (β/α, 6:4) of deacetylated trisaccharide **4h**[17] in 69% overall yield from **9** (average yield of 91% per step over four steps).

In summary, we have synthesized *O*-glycosyl trichloroacetimidates **1a**−**g** bearing Fmoc protected hydroxy groups in very good yields. Their application, for instance, of building block **1g**, to construct oligosaccharides in solution and on solid phase is very promising. For solid-phase oligosaccharide synthesis a novel ester-type linker was developed that allowed the construction of a trisaccharide in very good yield. Thus, the Fmoc group, which exhibits little sensitivity to acidic conditions, proved to be highly suitable for oligosaccharide synthesis on solid phase with ester-type linkers. We are currently developing the use of Fmoc-bearing building blocks to the synthesis of more complex oligosaccharides on solid phase.

Acknowledgment. We thank the European Community (grant FAIR-CT97-3142), the Bundesministerium für Bildung und Forschung (grant 0311 229), and the Deutsche Forschungsgemeinschaft for financial support of this work. We are grateful to Dr. A. Geyer for his help in the structural assignments and to C. Gege for the gift of a compound.

OL006081L

(16) (a) Windmüller, R. Diplomarbeit, Universität Konstanz, Germany, 1991. (b) Windmüller, R.; Schmidt, R. R. *Tetrahedron Lett.* **1994**, *35*, 7927.

(17) **Physical Properties of Compound 4h.** MALDI-TOF (DHB/THF) calcd $M(C_{75}H_{90}O_{16})$ = 1247.54 *m/z*, $(M + Na)^+$ = 1270.54 *m/z*, found 1270.4 *m/z*. **4hβ** (selected data): ^1H NMR (600 MHz, $CDCl_3$) δ 1.26 (3H, CH_3), 3.10 (5a-H), 3.29 (2a-H), 3.38 (5b-H), 3.39 (3a-H), 3.46 (6a-H), 3.53 (2c-H), 3.64 (6a-H′), 3.66 (4c-H), 3.70 (4b-H), 3.71 (3b-H), 3.73 (2b-H), 3.83 (3c-H), 3.89 (5c-H), 3.90 (4a-H), 4.20 (1a-H), 4.36 (1b-H), 5.44−5.45 (1c-H). **4hα** (selected data): ^1H NMR (600 MHz, $CDCl_3$) δ 3.34 (5b-H), 3.37 (2a-H), 3.42 (5a-H), 3.62 (3b-H), 3.70 (4b-H), 3.71 (2b-H), 3.86 (4a-H), 4.20 (1b-H), 4.60 (1a-H), 5.44−5.45 (1c-H).