Efficient Glycosylation Using ODS Adsorption Method Based on the Affinity of Long Alkoxybenzyl Glycoside

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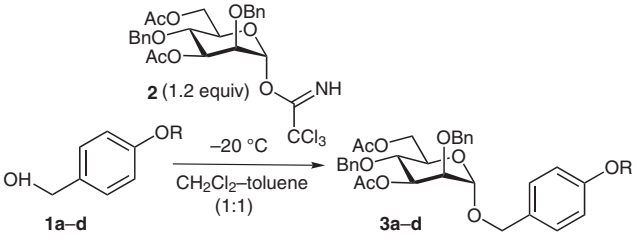
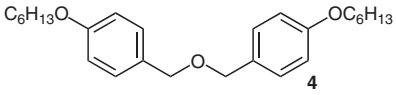
Abstract: *p*-Oleyloxybenzyl (POB) glycoside, selectively removable with TMSOTf, was developed as a protecting group for the glycosyl acceptor. Activation of the trichloroacetimidate was efficiently accomplished using 20 mol% of $Cu(OTf)_2$. Purification of the resulting glycoside was rapidly and efficiently accomplished by an ODS adsorption method based on the significant affinity of long alkyl chains and the ODS. The procedure is particularly useful for convergent oligosaccharide synthesis.

Key words: glycosylations, glycosides, octadecylsilane, *p*-oleyloxybenzyl ether, ODS adsorption

In recent years, the significance of sugar-chain engineering has grown rapidly based on an understanding of the importance of oligosaccharides in numerous biological events such as cell-to-cell interaction, recognition, inflammation, and viral infection.[1] Solid-supported oligosaccharide synthesis is one of the methods potentially useful in providing oligosaccharides at a practical level, and a variety of procedures have already been developed.[2,3] Recently, several methodologies incorporating the advantages of both solid- and liquid-phase syntheses, such as the capture-release strategy, and fluorous and ionic liquid tag methods have been reported.[4,5] In 2005, Rademann and co-workers introduced a novel hydrophobically assisted switching-phase method (HASP) for oligosaccharide synthesis based upon the affinity of a specially designed double-chain anchor with ODS (octadecylsilane).[6a] They carried out the glycosylation in the solution phase and following purification on the solid phase, the product, which adsorbed on ODS, was easily released by washing with CH_2Cl_2 for the next glycosylation in the solution phase. However, the hydrophobic anchor on the anomeric position is a benzyl ether derivative, and so can be removed only by catalytic hydrogenation (or Birch reduction). Thus the deprotection must be carried out at the final step in the oligosaccharide synthesis since in most cases common benzyl protecting groups are incorporated. Consequently, this method is not appropriate for the synthesis of the building blocks of a convergent strategy. Herein we describe selectively removable *p*-oleyloxybenzyl (POB) glycosides, which are useful both as the anomeric protection for glycosylation in the liquid phase, and also for the subsequent purification by ODS adsorption method, and will be very useful for convergent oligosaccharide synthesis.[7]



a: R = C_6H_{13}, **b**: R = $C_{12}H_{25}$, **c**: R = $(CH_2)_7CH{=}CH(CH_2)_8CH_3$ **d**: R = $C_{18}H_{37}$



Scheme 1 Synthesis of *p*-alkoxybenzyl glycosides

First we examined the glycosylation of D-mannosyl trichloroacetimidate **2** with *p*-hexyloxybenzyl alcohol (**1a**) to find the most suitable catalyst (Scheme 1).[8] Both TMSOTf and $BF_3{\cdot}OEt_2$ resulted in decomposition affording a complex mixture including bisbenzyl ether **4** (Table 1, entry 1 and 2). Although AgOTf did not act as catalyst, $Zn(OTf)_2$ afforded the glycoside **3a** in a quantitative yield with complete α-selectivity after 12 hours in CH_2Cl_2–toluene (1:1) at –20 °C (entries 3 and 4). The reaction using $Cu(OTf)_2$ (20 mol%) was completed within 15 minutes to give **3a** in quantitative yield also with complete α-selectivity (entry 5).[9] The CuOTf–benzene complex was also a good catalyst giving **3a** within one hour (entry 6). Glycosides **3b** and **3c** were also obtained by using $Cu(OTf)_2$ in excellent yields with complete α-selectivity (entries 7, 8). However, the glycoside **3d** was not obtained due to the poor solubility of **1d** under standard conditions (CH_2Cl_2–toluene, –20 °C, entry 9).

Next we investigated the purification of glycosides by ODS adsorption. Although the alkyl chain C_6H_{13} of **3a** as well as $C_{12}H_{25}$ of **3b** were not long enough to adsorb on an ODS column when eluted with MeCN, the glycoside **3c** was efficiently adsorbed on ODS by elution with MeCN. The adsorbed **3c** was cleanly recovered by elution with CH_2Cl_2. When the crude glycosylation product is transferred to the column of ODS, polar byproducts originated from the excess glycosyl donor should selectively wash out by elution with MeCN. Following elution with CH_2Cl_2, the desired glycoside should be released efficiently. Therefore, we decided to employ POB (*p*-oleyl-

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Table 1 Preparation of *p*-Alkoxybenzyl Glycosides

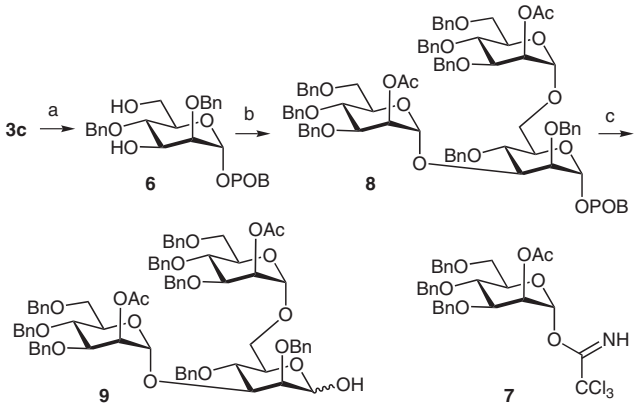
Entry	Benzyl alcohol	Catalyst[a]	Time (h)	Product	Yield (α/β)
1	**1a**	TMSOTf	0.25	**3a**	Trace (–)
2	**1a**	$BF_3{\cdot}OEt_2$	0.25	**3a**	Trace (–)
3	**1a**	AgOTf	12	**3a**	0 (–)
4	**1a**	$Zn(OTf)_2$	12	**3a**	99 (100:0)[b]
5	**1a**	$Cu(OTf)_2$	0.25	**3a**	99 (100:0)[b]
6	**1a**	CuOTf–benzene	1	**3a**	99 (100:0)[b]
7	**1b**	$Cu(OTf)_2$	0.25	**3b**	98 (100:0)[b]
8	**1c**	$Cu(OTf)_2$	0.25	**3c**	97 (100:0)[b]
9	**1d**	$Cu(OTf)_2$	0.25	**3d**	0 (–)

[a] The amount of 20 mol% was used.
[b] b-Isomer was not detected by ^{13}C NMR.

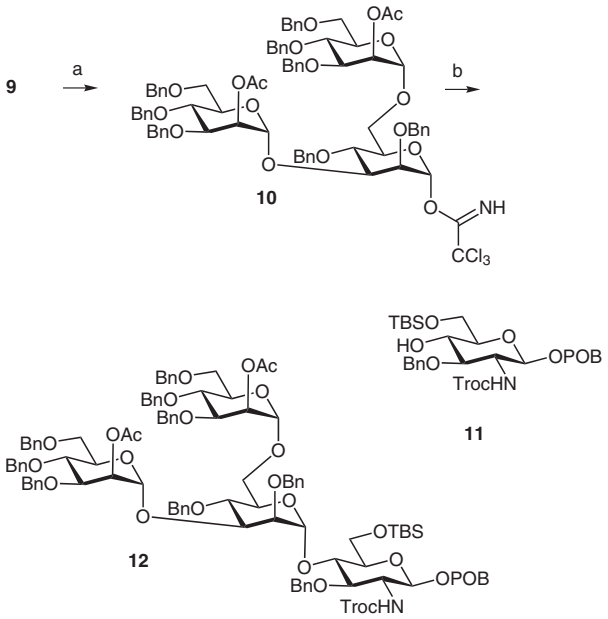
oxybenzyl) **1c** as the protecting group for the anomeric centers due to its high affinity for ODS.

In 2000, Eckhardt and co-workers reported the structure of *Clostridium botulinum* C2 toxin ligand **5** by analyzing the C2 toxin sensitivity of Chinese hamster ovary (CHO) cell mutants, which are deficient in specific sugar chains.[10] As segments of the N-linked oligosaccharide **5** would be useful in determining which parts of the sugar chain are essential for binding the C2 toxin, we tried to apply the present method to prepare a variety of segments of **5** (Figure 1).

Initially, the glycosyl acceptor **6** was prepared by the treatment of glycoside **3c** with NaOMe in MeOH. The glycosylation of **6** with an excess of **7** in CH_2Cl_2 was carried out using $Cu(OTf)_2$ (20 mol%) at 0 °C to give trisaccharide **8** in 95% yield with complete α-selectivity.[12] The impurities originating from the excess trichloroacetimidate **7** were efficiently and quickly removed by passing through an ODS column using MeCN, and trisaccharide **8** was obtained following elution with CH_2Cl_2.[11] The resulting **8** was pure enough to be used in the next reaction.[12] The *p*-oleyloxybenzyl group of **8** was selectively cleaved by treatment with 1.5 equivalents of TMSOTf in wet (H_2O sat.) toluene at 0 °C for 5 minutes giving rise to **9** in 72%



Scheme 2 Synthesis of trisaccharide **9**. *Reagents and conditions*: a, (i) NaOMe, MeOH, r.t., 1 h; (ii) ODS adsorption, 92%; b, (i) $Cu(OTf)_2$ (20 mol%), **7** (3.5 equiv), CH_2Cl_2, r.t., 12 h; (ii) ODS adsorption, 95%; c, TMSOTf (1.5 equiv), wet toluene, 0 °C, 5 min, 72%.



Scheme 3 Synthesis of tetrasaccharide **12**. *Reagents and conditions*: a, CCl_3CN (5 equiv), DBU (0.2 equiv), CH_2Cl_2, 0 °C, 3 h, 92%; b, (i) $Cu(OTf)_2$ (20 mol%), **11** (2 equiv), CH_2Cl_2, 0 °C, 4 h; (ii) ODS adsorption, 92%.

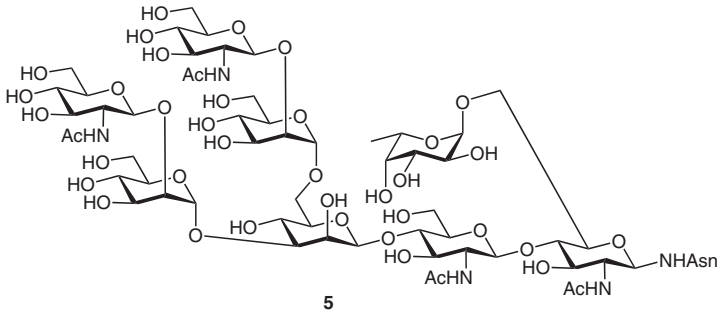
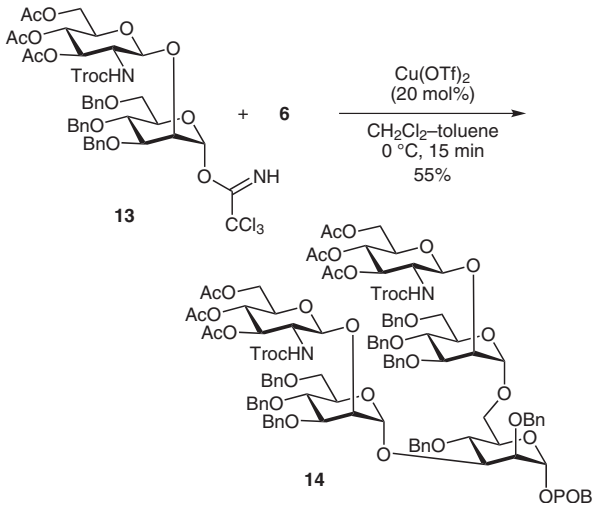
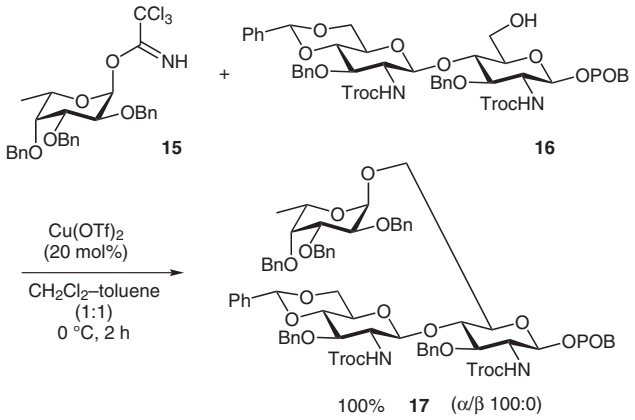


Figure 1 Structure of *Clostridium botulinus* C2 toxin ligand



Scheme 4 Synthesis of pentasaccharide **14**



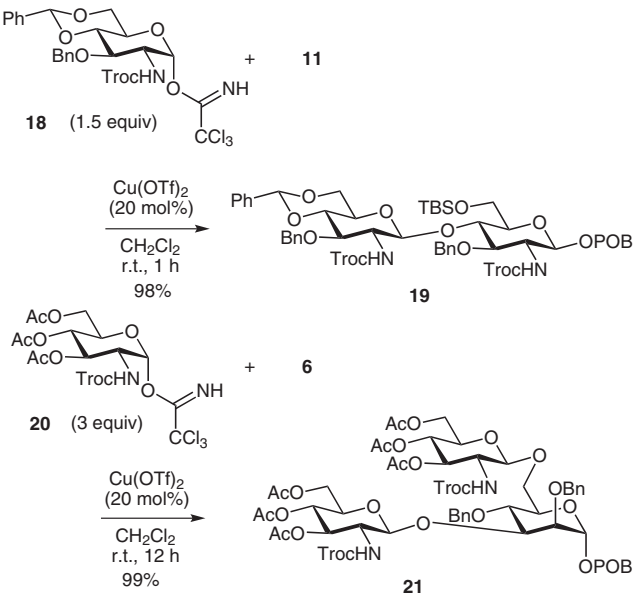
Scheme 5 Synthesis of α-core fucose-containing trisaccharide **17**

yield (Scheme 2).[13] The alcohol **9** was converted to **10** by treatment with CCl_3CN and DBU, and the glycosylation of **10** with **11** (Troc = 2,2,2-trichloroethoxycarbonyl) afforded a tetrasaccharide **12** in 92% yield with excellent α-selectivity (Scheme 3).[14] Purification of **12** was also efficiently achieved using the ODS adsorption method.

In contrast, the reaction of diol **6** with trichloroacetimidate **13** catalyzed by $Cu(OTf)_2$ afforded a mixture of stereoisomers of a pentasaccharide after quick purification by the ODS adsorption method. After further purification of the mixture by normal-phase HPLC, α,α-isomer **14** was obtained in 55% yield (Scheme 4).

The synthesis of the core fucose-containing segment **17** was successfully achieved by the glycosylation of **15** with **16** giving α-isomer **17** in a quantitative yield.[12] No trace of the β-isomer was detected by ^{13}C NMR after purification by the ODS adsorption method (Scheme 5). Chitobiose derivative **19** and trisaccharide segment **21** were also obtained in 98% and 99% yield, respectively, by using this methodology (Scheme 6).[12,15]

Therefore, we have developed an efficient glycosylation–purification procedure using an ODS adsorption method



Scheme 6 Synthesis of glucosamine derivatives **19** and **21**

based on the intense affinity of easily available long alkyl-chain protecting groups with ODS. Furthermore we synthesized segments of *Clostridium botulinum* C2 toxin ligand and demonstrated the usefulness of the present procedure for the construction of oligosaccharides in a convergent synthetic strategy.

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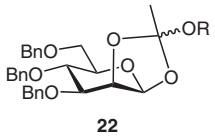
(11) **Synthesis of Trisaccharide 8 Using an ODS Adsorption Method – General Procedure**
To a stirred suspension of $Cu(OTf)_2$ (30 mg, 0.084 mmol) and 4 Å MS (powder, 50 mg) in anhyd CH_2Cl_2 (3 mL) was added a solution of diol **6** (300 mg, 0.42 mmol) at r.t., and the mixture was stirred for 20 min. To this was added a solution of trichloroacetimidate **7** (936 mg, 1.47 mmol) in CH_2Cl_2 (3 mL) using a syringe drive over a period of 1 h, and the mixture was stirred for 12 h at the same temperature. The reaction was terminated by the addition of Et_3N (720 mg, 7.12 mmol). Insoluble material was removed by passing through a cotton Celite pad, and the filtrate was concentrated under reduced pressure. The resulting material was dissolved in MeCN (100 mL) and adsorbed onto an ODS column (20 g), and the polar byproducts were eluted with MeCN (100 mL). Trisaccharide **8** (666 mg, 95% yield) was recovered by the elution with CH_2Cl_2.
Compound **8**: $[\alpha]_D^{22}$ +41.5 (*c* 1.9, $CHCl_3$). FT-IR (neat): 3087, 3062, 3004, 2925, 2855, 1951, 1878, 1809, 1745, 1611, 1585, 1511, 1496, 1454, 1368, 1285, 1237, 1132, 1101, 1050, 1026, 981, 913, 840, 736, 699, 604 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.89 (3 H, t, *J* = 6.8 Hz), 1.27–1.46 (22 H, m), 1.74–1.81 (2 H, m), 2.01–2.07 (4 H, m), 2.09 (3 H, s), 2.17 (3 H, s), 3.61–3.70 (3 H, m), 3.73–3.78 (2 H, m), 3.82–3.95 (11 H, m), 3.99 (1 H, dd, *J* = 9.2, 3.2 Hz), 4.03 (1 H, dd, *J* = 9.2, 3.2 Hz), 4.19 (1 H, dd, *J* = 9.6, 3.2 Hz), 4.31 (1 H, d, *J* = 11.6 Hz), 4.41–4.70 (12 H, m), 4.54 (1 H, d, *J* = 11.6 Hz), 4.76 (1 H, d, *J* = 11.2 Hz), 4.83 (1 H, d, *J* = 1.6 Hz), 4.88 (2 H, dd, *J* = 10.8, 3.2 Hz), 4.98 (1 H, d, *J* = 2.0 Hz), 5.20 (1 H, d, *J* = 1.6 Hz), 5.33–5.41 (2 H, m), 5.50–5.52 (2 H, m), 6.75–6.78 (2 H, m), 7.12–7.35 (42 H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.4 (q), 21.3 (q), 21.5 (q), 22.3 (t), 26.3 (t), 27.4 (t), 29.5–30.0 (many t), 32.2 (t), 32.9 (t), 66.7 (t), 68.2 (t), 68.7 (d, 2 C), 68.9 (t), 69.0 (d), 69.2 (t), 71.5 (d), 71.6 (d, 2 C), 72.0 (t), 72.4 (d), 72.4 (t), 73.6 (t), 73.6 (t), 74.4 (d), 74.5 (d), 75.2 (t), 75.3 (d), 75.4 (t), 77.5 (d), 77.8 (d), 77.9 (d), 78.3 (d), 79.0 (d), 95.7 (d), 98.2 (d), 99.9 (d), 114.6 (d), 127.7–130.2 (many d), 138.0 (s), 138.1 (s), 138.1 (s), 138.3 (s), 138.5 (s), 138.5 (s), 138.8 (s), 138.9 (s), 159.0 (s), 170.4 (s), 170.6 (s). MS (FAB, *m*-NBA): *m/z* = 1689 $[M + Na]^+$. HRMS (FAB): *m/z* calcd for $C_{103}H_{124}O_{19}Na$ $[M + Na]^+$: 1687.8635; found: 1687.8654.

(12) No other products were detected in the ^{13}C NMR spectrum of the saccharide.

(13) In the reaction using 2-acetylated trichloroacetimidate as glycosyl donor, an ortho ester such as **22** was produced as an intermediate. By continuing treatment with $Cu(OTf)_2$ (12 h), the ortho ester was transformed to the desired glycoside (Figure 2).



22

Figure 2

(14) The anomeric stereochemistry of **12** was determined from the coupling constants J_{CH} for anomeric carbons (164 Hz). Although the β-isomer was separable using HPLC, it was not possible to determine the stereochemistry by NMR spectroscopy due to insufficient sample. However, as the high-resolution mass spectrum suggested the structure of a tetrasaccharide, we concluded the stereochemistry is β.

(15) Compound **16** was prepared in 96% yield from the disaccharide **19** by treatment with HF·pyridine.