High-Efficiency Synthesis of Chitooligosaccharides

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Abstract: The solid-phase synthesis of chitooligosaccharides is described. After the *N*HCbz trichloroacetimidate donors **6** and **14** were synthesized; solid-phase synthesis was performed using the Wang resin as support. The illustrated tetra-*N*-acetyl-chitotetraose **1** was obtained by iterative glycosylation reactions, catalytic hydrogenation, acetylation, and deacetylation, respectively.

Keywords: Chitooligosaccharides, solid-phase synthesis, trichloroacetimidate donors, Wang resin, glycosylation reactions.

INTRODUCTION

Chitooligosaccharide (COS) is a kind of oligosaccharide which is made from chitin or chitosan by chemical or enzymatic decomposing method [1, 2]. COSs having *N*-acetyl analogues are of special interest in the agricultural and biomedical fields, because they exhibit strong bactericidal, fungicidal [3], and antitumour activities [4]. They also can be used as plant growth regulator [5].

The solid-phase synthesis is a rapid and efficient method to synthesize oligosaccharides [6, 7]. So, we also intend to prepare the chitooligosaccharides by solid-phase synthesis. It is easier to remove excess reactants or byproducts in the course of multi-step synthesis of chitooligosaccharides. Herein, the illustrated tetra-*N*-acetyl-chitotetraose **1** was synthesized by solid-phase method. The method is highly-efficient, and it can be used to synthesize other COSs.

RESULTS AND DISCUSSION

Treatment of α-D-glucosamine hydrochloride salt **2** with benzyloxycarbonyl (Cbz)-Cl in the presence of $NaHCO_3/H_2O$ yielded *N*-benzyloxycarbonyl protected glucosamine **3** in 88% yield. Acetylation of compound **3** by means of Ac_2O in pyridine obtained tetraacetate **4** as a mixture of α/β isomers in 4:1. The anomeric acetyl group was selectively removed using hydrazine acetate in DMF to afford hemiacetal **5**. Reaction of compound **5** with CCl_3CN in the presence of 1,8-diaza[5.4.0]bicycloundec-7-ene (DBU) exclusively afforded α-trichloroacetimidate donor **6** in 85% yield (Scheme **1**).

Treatment of compound **4** with hydrazine acetate in the presence of DMF obtained hemiacetal **7**, which was used without further purification. Then, the mixture was reacted with *tert*-butyldimethylsilyl (TBDMS)-Cl and imidazole to yield exclusively the β-anomer of the corresponding TBDMS derivative **8**. Deacetylation of compound **8** with NaOMe/MeOH afforded TBDMS 2-deoxy-*N*-benzyloxy-carbonylamino-β-D-glucopyranoside **9** in 96% yield. Treatment of compound **9** with benzaldehyde dimethylacetal afforded the 4,6-*O*-benzylidene derivative **10**. Compound **10** was treated with Ac_2O and pyridine to obtain acetate **11** in 94% yield. Regioselective reductive cleavage of benzylidene acetal **11** with CF_3COOH/Et_3SiH at 0°C afforded 6-*O*-Bn acceptor **12** in 85% yield. Compound **12** was treated with levulinic acid in the presence of *N,N'*-diisopropylcarbodiimide (DIPC) to yield the orthogonally protected glucosamine **13** in 94% yield. The anomeric TBDMS group was removed using tetrabutylammonium fluoride (TBAF) in the presence of acetic acid. Then, the crude product was reacted with CCl_3CN in the presence of DBU to afford the α-trichloroacetimidate donor **14** (Scheme **2**).

Glycosylation reactions were performed using 3.0 equiv. of donor and 1.2 equiv. of trimethylsilyl trifluromethanesulfonate (TMSOTf) as promoter for the activation of trichloroacetimidate donor. The Wang resin is a polymer support, and contains a linker [8]. At low temperature, TMSOTf promoted the glycosylation of trichloroacetimidate donor **14** with Wang resin, and the levulinoyl ester was cleaved using hydrazine acetate dissolved in MeOH to give the corresponding building block **15** in 86% yield (Scheme **3**). The yield was analyzed by high pressure liquid chromatography (HPLC) after cleavage of Wang resin with trifluoroacetic acid from building block **15**. Iterating the above process for two times obtained building block **16**. TMSOTf promoted the glycosylation of trichloroacetimidate donor **6** with building block **16** to give building block **17**. The resin was washed, filtered, and dried under the vacuum overnight. The saccharide bound resin was catalytically hydrogenated to cleave the Cbz, Wang resin, and Bn (about 91% yield). Then, the resulting mixture was acetylated with Ac_2O/pyridine and deacetylated with NaOMe/MeOH, respectively, to obtain a crude product. The crude product was purified by size-exclusion chromatography on Biogel P4 to afford the corresponding illustrated tetra-*N*-acetyl-chitotetraose **1** [9] in 77% yield for the last three steps. It indicated that the synthetic strategy for compound **1** was in reverse order for the installation of subunits **6** and **14**. In addition, due to the neighboring group participation of Cbz during the glycosylation reaction, the β-linkage is easy to form in compound **1**.

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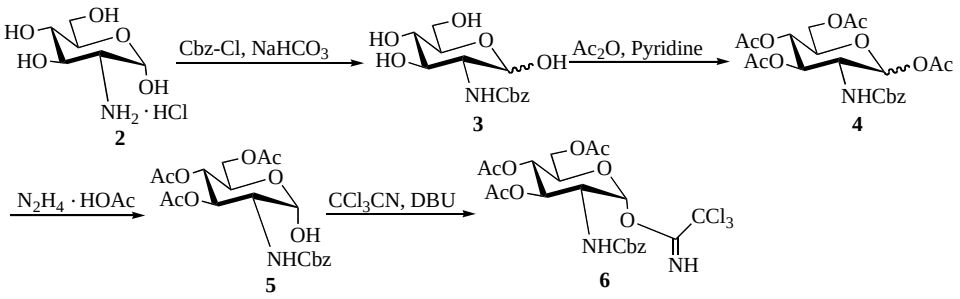
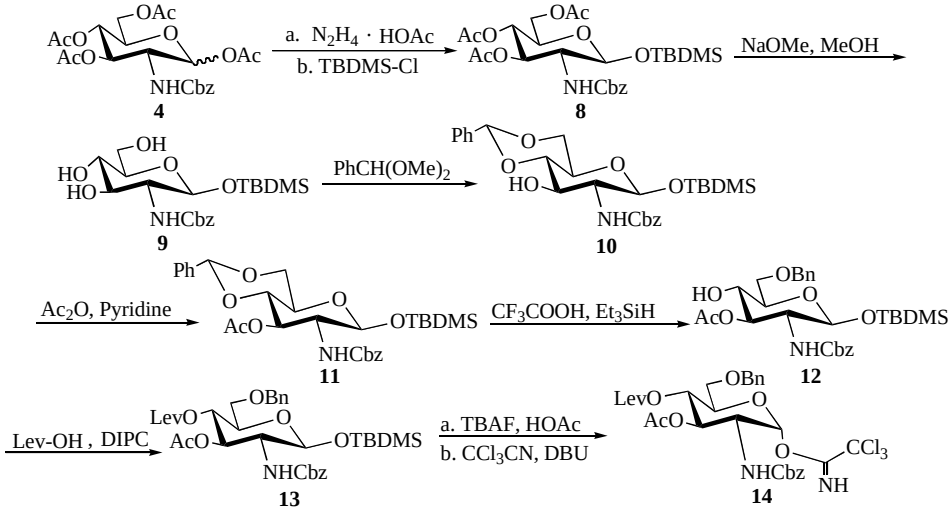
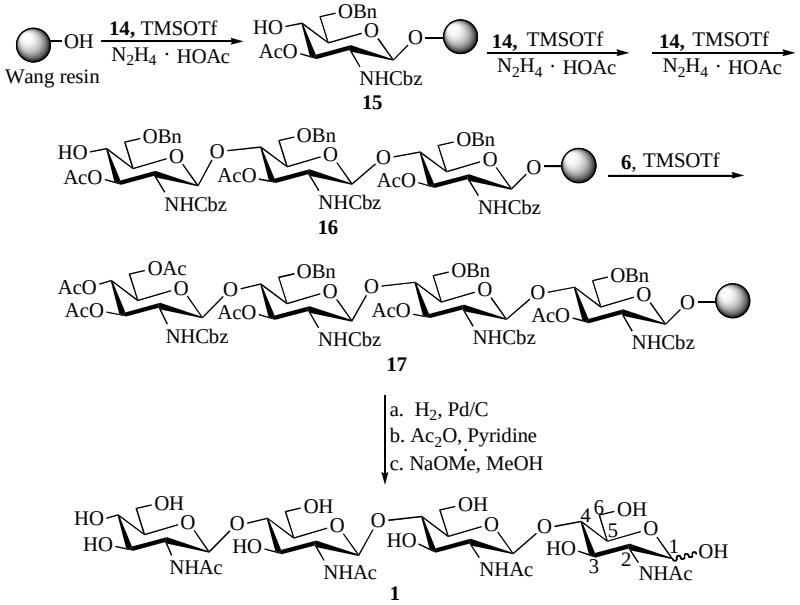


Scheme 1. Synthesis of *N*-Cbz protected donor **6**.



Scheme 2. Synthesis of *N*-Cbz-Donor **14**.



Scheme 3. Solid-phase synthesis of tetra-*N*-acetyl-chitotetraose **1**.

ACKNOWLEDGEMENTS

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[9] Tetra-*N*-acetyl-chitotetraose **1**: $[\alpha]_D$= -6.1 (*c*=0.5, H_2O). ^{13}C NMR (75MHz, D_2O): δ=177.00, 176.80 (C=O NHAc), 104.21, 103.81 (C-1^{II}, C-1^{III}, C-1^{IV}), 97.55 (β-C-1), 92.89 (α-C-1), 82.35-56.29 (C-2^{I-IV}, C-3^{I-IV}, C-4^{I-IV}, C-5^{I-IV}, C-$6a^{I-IV}$, C-$6b^{I-IV}$), 24.77, 24.63 (CH_3 NHAc). ^1H NMR (300MHz, D_2O): δ=5.16 (d, 0.6H, α-H-1), 4.69 (d, 0.4H, β-H-1), 4.55 (m, 3H, H-1^{II}, H-1^{III}, H-1^{IV}), 3.90-3.41 (m, 38H), 2.06, 2.02 (s, 12H, CH_3 NHAc). ESI-MS: *m*/*z*=853, $[M+Na]^+$.