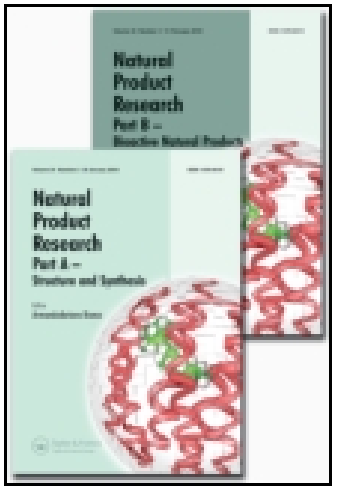

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Concise preparation of biologically active chitooligosaccharides

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Concise preparation of biologically active chitooligosaccharides

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Chitooligosaccharides (COSs) have demonstrated a diverse array of biological activities. Here we report a concise preparation method for tetra-*N*-acetyl-chitotetraose and penta-*N*-acetyl-chitopentaose. The FACE analysis showed that the partially *N*-acetylated COS mixture mainly contained glucosamine (GlcN) and some oligomers [$(GlcN)_n$, $n = 2$–7]. The *N*-acetyl-D-glucosamine (GlcNAc) and peracetylated COSs [$(GlcNAc)_n$, $n = 2$–7] were synthesised by treating the partially *N*-acetylated COS mixture with Ac_2O–NaOAc. The peracetylated chitotetraose and chitopentaose were obtained by isolation of peracetylated COS mixture. NaOMe in dry MeOH was used for the deacetylation of peracetylated chitotetraose and chitopentaose, to give the tetra-*N*-acetyl-chitotetraose and penta-*N*-acetyl-chitopentaose, respectively.

Keywords: chitooligosaccharides; tetra-*N*-acetyl-chitotetraose; penta-*N*-acetyl-chitopentaose; preparation

1. Introduction

Chitosan is a linear heteropolysaccharide composed of β-1,4-linked-D-glucosamine (GlcN) and *N*-acetyl-D-glucosamine (GlcNAc) in varying proportions. Chitooligosaccharides (COSs) are known to be easily prepared by acidic or enzymatic partial hydrolysis of chitosan. It has also been reported that lower COSs are water soluble and biologically active (Huang, Mei, Zhang, & Wang, 2006; Suzuki et al., 1986). Furthermore, the biological activity of COSs is known to depend on their structures (Hahn, 1996). COSs having *N*-acetyl analogues are of special interest in the agricultural and biomedical fields, because they exhibit strong bactericidal, fungicidal (Hirano & Nagao, 1989) and antitumour activities (Suzuki et al., 1986).

Herein, we prepared and characterised the tetra-*N*-acetyl-chitotetraose and penta-*N*-acetyl-chitopentaose using a partially *N*-acetylated COS mixture as starting material. The method is simple, and it can be used to prepare the other pure peracetylated COS.

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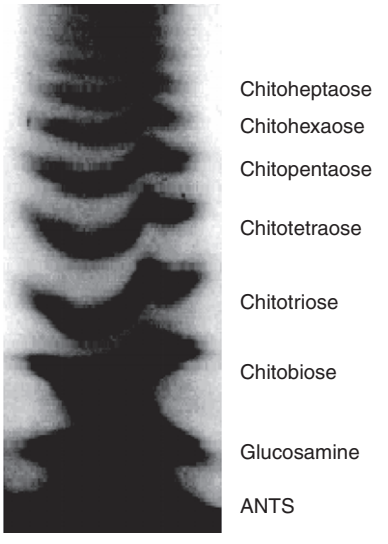

Figure 1. FACE of partially *N*-acetylated COS mixture.

2. Results and discussion

2.1. *FACE analysis of partially* N-*acetylated COS mixture*

Fluorophore-assisted carbohydrate electrophoresis (FACE) is a high-resolution polyacrylamide gel electrophoretic procedure that separates oligosaccharides on the basis of size (Huang, 2008; Huang, Mei, & Wang, 2006; Huang & Wong, 2008). The partially *N*-acetylated COS mixture was tagged at the terminal aldehyde with the highly charged fluorophore 8-aminonaphthalene-1,3,6-trisulfonate (ANTS). Then, the saccharide-ANTS adducts were separated on 32% C_{ACR}/2.4% C_{BIS} (Figure 1) polyacrylamide gels at alkaline pH value. It was indicated that the partially *N*-acetylated COS mixture mainly contained GlcN and some oligomers $[(GlcN)_n, n = 2–7]$.

2.2. *Preparation of tetra-*N-*acetyl-chitotetraose and penta-*N-*acetyl-chitopentaose*

The partially *N*-acetylated COS mixture was acetylated with Ac_2O–NaOAc to maximise the yield of peracetylated COSs. The peracetylated COS mixture was isolated on a silical gel 60 column to give peracetylated chitotetraose and peracetylated chitopentaose, respectively. The peracetylated chitotetraose and chitopentaose were deacetylated with NaOMe in dry MeOH at room temperature, sequentially neutralised, desalted and evaporated, to give the corresponding tetra-*N*-acetyl-chitotetraose and penta-*N*-acetyl-chitopentaose in the overall yields of 16 and 10%, respectively (Figure 2).

3. Experimental

3.1. *General*

Partially *N*-acetylated COS mixture was purchased from the Aoxing Biotechnology Co., Ltd. (Zhejiang Province, China). *N*,*N*′-methylenebisacrylamide,

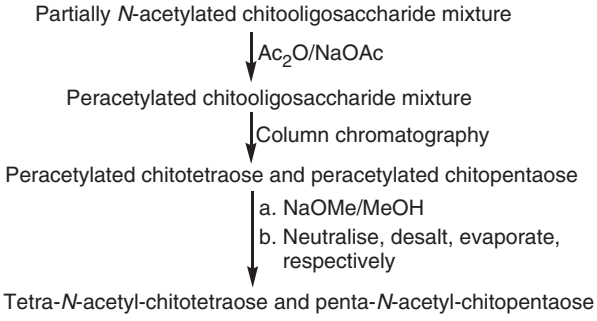

Figure 2. Preparation of tetra-*N*-acetyl-chitotetraose and penta-*N*-acetyl-chitopentaose.

N,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), ammonium persulfate, 8-amino-naphthalene-1,3,6-trisulfonate (ANTS) and sodium cyanoborohydride were purchased from Sigma-Aldrich Chemical Company. Optical rotations were determined at 25°C with a Perkin–Elmer Model 241-Mc automatic polarimeter. ^{13}C and ^{1}H NMR spectra were recorded using a Bruker DPX-300 spectrometer at 75 and 300 MHz, respectively. Mass spectra were recorded with a VG PLATFORM mass spectrometer using the ESI mode. TLC was performed on silica gel plates (GF_{254}) with detection by UV light or by charring with 10% H_2SO_4 in EtOH.

3.2. *Electrophoresis of ANTS-labelled partially* N*-acetylated COS mixture*

The resolving gel was 32% acrylamide/2.4% bisacrylamide in a 140 × 160 × 0.75 mm glass cassette. For every 35 mL of resolving gel, 150 μL of 10% ammonium persulphate and 15 μL of TEMED were added. The stacking gel was 8% acrylamide/0.6% bisacrylamide containing 50 and 5 μL of ammonium persulphate and TEMED, respectively, for every 6 mL of stacking gel. The running buffer and the gel buffer were 0.025 mol L^{-1} Tris/0.192 mol L^{-1} glycine (pH 8.4) and 0.42 mol L^{-1} Tris (pH 8.5), respectively. Electrophoresis was run at a constant current of 15 mA for 6 h in a cooled buffer system.

3.3. *Preparation of target compounds*

To boiling Ac_2O (20 mL) in a three-necked flask, 200 mg of KOAc was added. Then, 200 mg of partially *N*-acetylated COS mixture was gradually added under vigorous stirring. The solution was kept for 1 h at 140°C, then cooled to room temperature. The crude product directly was purified on a silical gel 60 column (eluant : AcOEt : EtOH 4 : 1), and the peracetylated chitotetraose and peracetylated chitopentaose were obtained, respectively. The peracetylated chitotetraose or peracetylated chitopentaose were suspended in anhydrous MeOH to a concentration of 100 mg mL^{-1} and deacetylated with an equal volume of 1 mol L^{-1} NaOMe at room temperature for 60 min with continuous mixing. The sample was then desalted and neutralised by addition of Amberlite IRC-50 (H^+) ion-exchange resin (40 mg mg^{-1} sample) previously washed in MeOH. After 30 min of vigorous shaking, the deacetylated sample was recovered and the IRC-50 ion exchanger was

washed with MeOH. Then, the combined washings were evaporated to dryness under reduced pressure at 45°C. The tetra-*N*-acetyl-chitotetraose and penta-*N*-acetyl-chitopentaose were obtained in the overall yields of 16 and 10%, respectively. Tetra-*N*-acetyl-chitotetraose, $[\alpha]_D = -6.1$ ($c = 0.5, H_2O$). ^{13}C NMR (75 MHz, D_2O): $\delta = 177.00$, 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): $\delta = 5.16$ (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: $m/z = 853$, $[M + Na]^+$. Penta-*N*-acetyl-chitopentaose, $[\alpha]_D = -5.7$ ($c = 0.5, H_2O$). ^{13}C NMR (75 MHz, D_2O): $\delta = 175.57$, 175.40 (C=O NHAc), 102.39, 102.26 (C-1^{II}, C-1^{III}, C-1^{IV}, C-1^{V}), 95.75 (β-C-1), 91.39 (α-C-1), 80.65–54.60 (C-$2^{I–V}$, C-$3^{I–V}$, C-$4^{I–V}$, C-$5^{I–V}$, C-$6a^{I–V}$, C-$6b^{I–V}$), 23.09, 22.90 (CH_3 NHAc). ^1H NMR (300 MHz, D_2O): $\delta = 5.10$ (d, 0.7H, α-H-1), 4.61 (d, 0.3H, β-H-1), 4.44 (m, 4H, H-1^{II}, H-1^{III}, H-1^{IV}, H-1^{V}), 3.89–3.30 (m, 47H), 1.96, 1.93 (s, 15H, CH_3 NHAc). ESI-MS: $m/z = 1056$, $[M + Na]^+$.

4. Conclusion

Tetra-*N*-acetyl-chitotetraose and penta-*N*-acetyl-chitopentaose were obtained in the process of acetylation, column chromatography, deacetylation, neutralisation, desalting and evaporation of partially *N*-acetylated chitooligosaccharide mixture, respectively.

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