

Short Note

Chitooligosaccharide-2,5-anhydro-D-mannonic Acid

Emil Salim, Daniela Ailincai and Stéphane Trombotto *

Ingénierie des Matériaux Polymères (IMP, UMR CNRS 5223), Université Claude Bernard Lyon 1, Université de Lyon, Villeurbanne F-69622, France

* Author to whom correspondence should be addressed; E-Mail: stephane.trombotto@univ-lyon1.fr; Tel.: +33-4-7243-1210; Fax: +33-4-7243-1249.

Received: 26 June 2014 / Accepted: 14 August 2014 / Published: 19 August 2014

Abstract: Chitooligosaccharide with one 2,5-anhydro-D-mannofuranose unit at the reducing end (COS_{amf}) was prepared by nitrous deamination of fully *N*-deacetylated chitosan. The oxidation of the amf-unit by chlorite sodium was achieved in high yield. The chemical structure of the targeted chitooligosaccharide-2,5-anhydro-D-mannonic acid with a degree of polymerization ~10 was fully characterized by NMR spectroscopy, MALDI-TOF mass spectrometry and size-exclusion chromatography. The success of this synthesis opens the way to a new generation of end-functionalized COS building blocks.

Keywords: chitosan; chitooligosaccharide-2,5-anhydro-D-mannofuranose; 2,5-anhydro-D-mannonic acid; nitrous deamination; oxidation

Chitosan is a linear polysaccharide of D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) units linked by β -(1→4) glycosidic bonds. Chitosan is produced industrially by alkaline *N*-deacetylation of chitin, the second most abundant naturally occurring polymer, mainly present in the exoskeleton of arthropods and in the endoskeleton of cephalopods [1,2]. Chitosan and chitin oligomers, also named chitooligosaccharides (COS), have recently received considerable attention as functional biomolecules with a wide range of applications in food, agriculture, medicine, pharmaceuticals and cosmetics. COS take advantage of their various remarkable physico-chemical and biological properties, including water-solubility, biocompatibility, antibacterial, antifungal and antitumoral activities [3,4,5,6].

Among various chemical methods described in the literature for the preparation of COS by depolymerisation of chitin or chitosan, the nitrous acid (HNO₂) deamination methodology presents several advantages: (i) the reaction is selective and rapid; (ii) the reaction can be carry out in

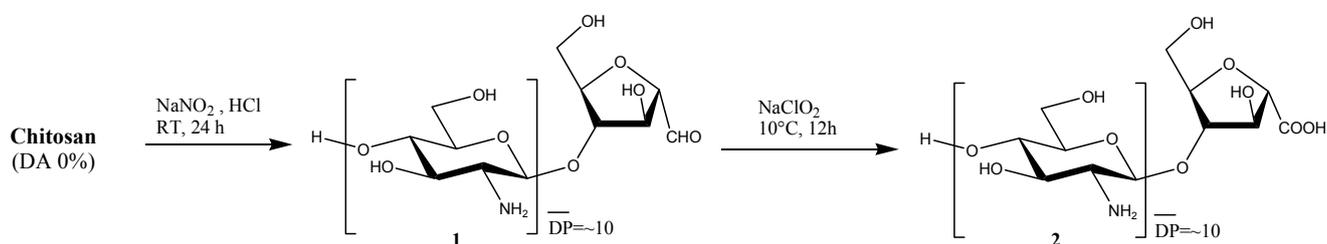
homogeneous aqueous solutions and mild reaction conditions of temperature and pH; (iii) the extent of depolymerisation can be controlled through the stoichiometry of reaction [7,8,9]. The mechanism has been found to be specific in the sense that HNO_2 attacks the amino group of GlcN-units, with a subsequent cleavage of the following glycosidic linkage and a formation of 2,5-anhydro-D-mannofuranose (amf-unit) at the position corresponding to the reducing end. Because the amf-residue in COS is rather unstable in mild acidic conditions, due to its decomposition to hydroxymethylfurfural (HMF), the standard procedure has been its reduction to 2,5-anhydro-D-mannitol by NaBH_4 [10,11].

In order to develop a new easy access of stable COS from nitrous deamination of chitosan, the oxidation of the aldehyde group of amf-units in COS has been studied. We describe here the synthesis and the characterisation of chitooligosaccharide-2,5-anhydro-D-mannonic acid with a low degree of polymerization (DP) ~ 10 by reaction of COSamf with sodium chlorite. Furthermore, the acid group of 2,5-anhydro-D-mannonic acid could be available for reactions that could make the obtained COS an interesting precursor in organic synthesis.

Results and Discussion

Chitooligosaccharide-2,5-anhydro-D-mannonic acid with an average GlcN-units number into chains around 10, was efficiently synthesized from chitosan in a one pot two-step procedure involving the aldehyde group oxidation of the intermediate chitooligosaccharide-2,5-anhydro-D-mannofuranose (COSamf **1**) with sodium chlorite as illustrated in Scheme 1.

Scheme 1. Synthesis of chitooligosaccharide-2,5-anhydro-D-mannonic acid (**2**) from chitosan.



Thus, COSamf **1** was produced by nitrous acid deamination of a fully *N*-deacetylated chitosan as described in a previous study [12]. Briefly, the depolymerization of chitosan (DA 0%, $M_w = 270$ kg/mol; $M_n = 115$ kg/mol, $\bar{D} = 2.3$) was carried out using sodium nitrite (GlcN/ NaNO_2 molar ratio = 4) in aqueous acid solution at room temperature. After 24 h of reaction, COSamf **1** could be isolated and purified in 80% mass yield by both ultrafiltration and precipitation in ethanol. The chemical structure of COSamf **1** was fully confirmed by ^1H and ^{13}C -NMR spectroscopies, MALDI-TOF mass spectrometry and size-exclusion chromatography (see Supporting Information). Therefore it has been shown COSamf **1** is composed of a mixture of GlcN-unit oligomers, with an average DP around 10.

In a second step, the selective oxidation of the amf-unit aldehyde group by sodium chlorite has been directly carried out after the nitrous deamination step at 10 °C for 12 h, leading to the targeted chitooligosaccharide-2,5-anhydro-D-mannonic acid **2** in a good mass yield (70%) from chitosan, after purification by ultrafiltration and precipitation in ethanol. The chemical structure of the compound **2** was fully characterized by ^1H and ^{13}C -NMR spectroscopies thanks to two-dimensional NMR analyses, pointing out the loss of the aldehyde group at 5.1 ppm in the ^1H -NMR spectrum and the formation of

the carboxylic group at 175.0 ppm in the ^{13}C -NMR spectrum [13]. As confirmed by MALDI-TOF mass spectrometry, chitooligosaccharide-2,5-anhydro-D-mannonic acid **2** is composed of a mixture of GlcN-unit oligomers (see Supporting Information). The average number of GlcN units into chains was determined by both ^1H -NMR and SEC and was found to be equal to around 10, as for COSamf **1**. Additionally, it has been shown this oxidation procedure can be carried out on COSamf with higher average GlcN-units number into chains, *i.e.* DP \sim 20 and \sim 30, leading to corresponding chitooligosaccharide-2,5-anhydro-D-mannonic acids in high mass yields from chitosan, respectively 80% and 90%.

As expected, chitooligosaccharide-2,5-anhydro-D-mannonic acid **2** has shown a good stability in both aqueous solution and powder form at low to room temperatures over several months. Thus, no decomposition into HMF residues was observed in ^1H -NMR analyses, contrarily to what could be shown for COSamf **1** in same storage conditions.

Experimental

Chitooligosaccharide-2,5-anhydro-D-mannonic acid 2: A fully *N*-deacetylated chitosan (2.1 g, 13 mmol GlcN unit) was solubilized in 100 mL of water by addition of 1.15 mL HCl (37% w/w). Then, a freshly prepared 5 mL aqueous solution of NaNO_2 (0.66 M) was added and the reaction was allowed to proceed for 24 h at room temperature. For isolation and characterisation of COSamf **1**, the solution was neutralised (pH 7–8) by 1M sodium hydroxide, then ultra-filtered (cellulose ester membrane, MWCO 500, Spectrum Labs). COSamf **1** was obtained as a white powder after precipitation in ethanol and drying under vacuum (1.7 g, 80% mass yield). For the oxidation step, a 30 mL aqueous solution of sodium chlorite (0.45 M) was added at 10 °C to the COSamf **1** solution obtained after the nitrous deamination step, and the reaction was stirred for 12 h at RT. The solution was neutralised (pH 7–8) by sodium hydroxide, then ultra-filtered (cellulose ester membrane, MWCO 500 g/mol, Spectrum Labs). The product was precipitated in ethanol and drying under vacuum leading to compound **2** as white powder (1.5 g, 70% total mass yield). ^1H -NMR (300 MHz, D_2O , 298 °K): δ (ppm) 4.70 (m, H-3 amf), 4.58 (d, $J = 3.0\text{Hz}$, H-2 amf), 4.30 (m, H-5 amf), 4.22 (m, H-4 amf), 4.00–3.40 (m, H-3 to H-6 GlcN, H-6 amf), 3.18 (m, H-2 GlcN). ^{13}C -NMR (125 MHz, D_2O , 298 °K): δ (ppm) 175.0 (COOH), 99.1 (C-1' GlcN), 98.2 (C-1 GlcN), 86.0 (C-4 amf), 84.9 (C-5 amf), 82.8 (C-2 amf), 79.3 (C-3 amf), 77.1 (C-5' GlcN), 77.0 (C-4 GlcN), 75.5 (C-5 GlcN), 72.4 (C-3' GlcN), 70.7 (C-3 GlcN), 70.3 (C-4' GlcN), 61.8 (C-6 amf), 61.0 (C-6' GlcN), 60.7 (C-6 GlcN), 56.6 (C-2 GlcN), 56.3 (C-2' GlcN). *Note that C' represents carbon atoms of the GlcN unit linked to the amf unit.* MALDI-TOF MS: presence of a major peak at m/z 990.5 attributed to $\text{HO}-(\text{GlcN})_5-\text{C}_6\text{H}_9\text{O}_4$ (m/z monoisotopic calcd for $[\text{C}_{36}\text{H}_{65}\text{O}_{25}\text{N}_5\text{Na}]^+ = 990.4$ mass units ($\Delta = 0.01\%$)). HRMS (ESI): calcd for $\text{C}_{36}\text{H}_{65}\text{O}_{25}\text{N}_5\text{Na}$: m/z 990.3866; found 990.3850 $[\text{M}+\text{Na}]^+$ (difference = 1.6 ppm).

Acknowledgments

The authors thank Catherine Ladavière (IMP, CNRS), Agnès Crepet (IMP, CNRS) and Bernard Fenet (CCRMN, Université Lyon 1) for their helpful assistance and discussions in MALDI-TOF mass spectrometry, size-exclusion chromatography and NMR spectroscopy analyses, respectively. Financial support from the University Lyon 1 is greatly acknowledged.

Author Contributions

All the authors equally contributed to the research. ES and DA did the experiments. ST performed the spectroscopy data and wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

References and Notes

1. Dash, M.; Chiellini, F.; Ottenbriteb, R.M.; Chiellini, E. Chitosan: A versatile semi-synthetic polymer in biomedical applications. *Prog. Polym. Sci.* **2011**, *36*, 981–1014.
2. Yeul, V.S.; Rayalu, S.S. Unprecedented Chitin and Chitosan: A Chemical Overview. *J. Polym. Environ.* **2013**, *21*, 606–614.
3. Aam, B.B.; Heggset, E.B.; Norberg, A.L.; Sørli, M.; Vårum, K.M.; Eijsink, V.G.H. Production of chitooligosaccharides and their potential applications in medicine. *Mar. Drugs* **2010**, *8*, 1482–1517.
4. Xia, W.; Liu, P.; Zhang, J.; Chen, J. Biological activities of chitosan and chitooligosaccharides. *Food Hydrocolloids* **2011**, *25*, 170–179.
5. Lodhi, G.; Kim, Y.-S.; Hwang, J.-W.; Kim, S.-K.; Jeon, Y.-J.; Je, J.-Y.; Ahn, C.-B.; Moon, S.-H.; Jeon, B.-T.; Park, P.-J. Chitooligosaccharide and Its Derivatives: Preparation and Biological Applications. *BioMed Res. Int.* **2014**, *2014*, doi:10.1155/2014/654913.
6. Trombotto, S.; Ladavière, C.; Delolme, F.; Domard, A. Chemical preparation and structural characterization of a homogeneous series of chitin/chitosan oligomers. *Biomacromolecules* **2008**, *9*, 1731–1738.
7. Tommeraas, K.; Varum, K.M.; Christensen, B.E.; Smidsrod, O. Preparation and characterization of oligosaccharides produced by nitrous acid depolymerization of chitosans. *Carbohydr. Res.* **2001**, *333*, 137–144.
8. Hussain, I.; Singh, T.; Chittenden, C. Preparation of chitosan oligomers and characterization: their antifungal activities and decay resistance. *Holzforschung* **2012**, *66*, 119–125.
9. Mourya, V.K.; Inamdar, N.N.; Choudhari, Y.M. Chitooligosaccharides: Synthesis, characterization and applications. *Polym. Sci. Ser. A Polym. Phys.* **2011**, *53*, 583–612.
10. Sashiwa, H.; Saimoto, H.; Shigemas, Y.; Tokura, S. Chitooligosaccharides: Synthesis, characterizations and applications. *Carbohydr. Res.* **1993**, *242*, 167–172.
11. Lin, C.-W.; Lin, J.-C. Characterization and blood coagulation evaluation of the water-soluble chitooligosaccharides prepared by a facile fractionation method. *Biomacromolecules* **2003**, *4*, 1691–1697.
12. Salim, E.; Galais, A.; Trombotto, S. 4-(Hexyloxy)aniline-linked chitooligosaccharide-2,5-anhydro-D-mannofuranose. *Molbank* **2014**, *2014*, M815.
13. Note that in the ¹H-NMR analysis conditions used here, the aldehyde group of the amf-units does not exist in its free (-CHO) form but exclusively in its hydrated (-CH(OH)₂) form.