Carbohydrate Polymers 145 (2016) 37–47

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis and characterization of new chitosan-based Schiff base compounds

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ARTICLE INFO

Article history:
Received 18 December 2015
Received in revised form 23 February 2016
Accepted 28 February 2016
Available online 10 March 2016

Keywords:
Chitin
Chitosan
4-(2-Haloethyl)benzaldehyde
New chitosan Schiff base

ABSTRACT

Chitin (Cn) was extracted from the armors of crustaceans *Astacus leptodactylus* (Lake Sevan, Armenia) and then converted to chitosan (Cs), its deacetylated derivative. Novel Schiff bases (CsSB) were synthesized by interaction of Cs with 4-(2-chloroethyl)benzaldehyde (aldehyde-1) and 4-(2-bromoethyl)benzaldehyde (aldehyde-2), and underwent dehydrohalogenation, under basic conditions (10 °C), to yield respective vinyl derivatives. All newly synthesized compounds were structurally characterized by solubility tests, elemental analysis, infrared spectroscopy (FTIR), thermogravimetry (TGA), proton nuclear magnetic resonance (^{1}H NMR), and X-ray diffraction (XRD).

1. Introduction

Chitosan (Cs) [β-(1 → 4)-2-amino-2-deoxy-D-glucopyranose] is the product of deacetylation of chitin (Cn) [β-(1 → 4)-2-acetamido-2-deoxy-D-glucopyranose]. Some products of Cs chemical transformations are biocompatible, biodegradable and non-toxic; they exhibit antioxidant properties and have been widely used in biomedicine (Kumirska, Weinhold, Thöming, & Stepnowski, 2011), environmental protection (Kocak, Sahin, Arslan, & Ucan, 2012), agriculture (Hadrami, Adam, Hadrami, & Daayf, 2010), and textile industry (Klaykruayat, Siralertmukul, & Srikulkit, 2010). Numerous derivatives of Cs were synthesized due to the pending amino and hydroxyl groups peripherially attached to the macromolecule backbone (Mourya, Inamdar, & Tiwari, 2010; Santos, Dockal, & Cavalheiro, 2005; Sajomsang, Tantayanon, Tangpasuthadol, Thatte, & Daly, 2008). The Schiff bases that can be obtained by interaction of the Cs amino groups with aldehydes and ketones are of immediate interest as biomaterials endowed with a set of unique properties (Jiao et al., 2011; Sashiwa & Aiba, 2004). In particular, the newly formed imino groups increase the physiological activity of chitosan Schiff bases (CsSB), so that they can act as antibacterial (Soliman, El-Kousy, Abd-Elbary, & Abou-zeid, 2013), antitumor (Park, Chung, Choi, & Park, 2011), antimicrobial and anticorrosive (Mohamed & Fekry, 2011) agents. CsSB were also synthesized with aromatic aldehydes as carbonyl components (Jiao et al., 2011; Santos et al., 2005; Sajomsang et al., 2008). The existence of electron-donating and electron-withdrawing substituents at the aromatic ring exerted a noticeable influence on the electrophilicity of the carbonyl group and on the degree of substitution (DS) of CsSB. As a carbonyl compound, to the best of our knowledge, 4-(2-chloroethyl) benzaldehyde (aldehyde-1) and 4-(2-bromoethyl) benzaldehyde (aldehyde-2) have not been used in the CsSB synthesis. Structurally, an attractive feature in aldehydes chosen is the presence of a XCH_2CH_2-group that allows for elimination reaction and for introducing an unsaturation in the select positions of the molecular assembly. In particular, pending vinyl groups would allow for the synthesis of new polymeric systems with potentially valuable properties. Herein we report on the synthesis of new CsSB derivatives and their structural characterization by means of TGA, ^{1}H NMR, FTIR, and XRD as well as elemental analysis.

Abbreviations: Cn, chitin; Cs, chitosan; CsSB, chitosan Schiff base; Aldehyde-1, 4-(2-chloro)ethylbenzaldehyde; Aldehyde-2, 4-(2-bromo)ethylbenzaldehyde; FTIR spectroscopy, Fourier-Transformed Infrared spectroscopy; TGA, thermogravimetry; ^{1}H NMR, proton nuclear magnetic resonance; XRD, X-ray diffraction; DRON-3, general purpose X-ray diffractometer-3; DS, degree of substitution; ppm, parts per million; CsSBRTCl, CsSB from aldehyde-1 synthesized at RT; CsSB80Cl, CsSB from aldehyde-1 synthesized at 80 °C; CsSBRTBr, CsSB from aldehyde-2 synthesized at RT; CsSB80Br, CsSB from aldehyde-2 synthesized at 80 °C; CsSBVinyl-1, vinyl derivative from CsSBRTCl; CsSBVinyl-2, vinyl derivative from CsSBRTBr; TG, thermogravimetric; DTG, derivative thermogravimetric; DTA, differential thermal analysis; X, halogen.

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http://dx.doi.org/10.1016/j.carbpol.2016.02.076
0144-8617/

2. Experimental

2.1. Materials and methods

Reagents and solvents were purchased from various vendors and used without further purification. Sodium hydroxide, potassium hydroxide, hydrochloric acid, sulfuric acid, glacial acetic acid, acetone, diethyl ether, methanol, ethanol, benzene, chloroform, dichloromethane, tetrahydrofuran, *N,N*-dimethylformamide, silver nitrate, and dimethyl sulfoxide were purchased from Reahim (chemically pure), tetrachloromethane and trifluoroacetic acid were delivered by Sigma-Aldrich, and sodium bicarbonate, hexamethylentetramine, magnesium sulfate, aqueous ammonia, and sodium borohydride were obtained from Reahim (pure for analysis). Deiterium oxide, trifluoroacetic acid-d, and dimethyl sulfoxide-d_6 were acquired from Cambrige Isotope Laboratories (Andover, MA). In the solubility tests, the ratio of the product mass (mg) and a volume of solvent (mL) was equal to 1: 2. Elemental analyses (C, H, N) were performed on Euro EA 3000 analyzer (Italy). Melting temperatures were measured using a Boetius tipe microscope with a heating stage. Attenuated total reflection Fourier-transform infrared spectra (FTIR ATR) were registered by Nicolet 5700 spectrometer (ZnSe prism, Happ-Genzel apodization, correction of ATR distortion, number of scans 32, resolution 4 cm^{-1}). The thermogravimetry (TGA) was carried out in air atmosphere using Derivatograph Q-1500 (Hungary; temperature gradient: room temperature to 700 °C/10 °C/min). The initial weight of each sample was 100 mg. The proton nuclear magnetic resonance (^{1}H NMR) spectra were measured using Varian Mercury 300VX 300 MHz spectrometer at 303 K. X-ray diffraction (XRD) measurements were conducted using DRON-3 X-ray diffractometer with CuKα radiation (2θ, 10–80°).

2.2. Cn extraction

Armors of crayfish *Astacus leptodactylus* (Lake Sevan, Armenia) were cleaned of pectins, grinded to a fine powder, and extracted according to the literature protocol (Lertsutthiwong, How, Chandrkrachang, & Stevens, 2002; "Process 2"). Demineralization was carried out with dilute HCl (3.5% HCl; powder mass to acid volume (w/v) was equal to 1:14) at RT for 2.5 h. Under continuous stirring, the acid solution was added portionwise until the release of carbon dioxide ceased off completely. The product was washed with distilled water until no chloride ions remained in the solution ($AgNO_3$ test), then dried at 60 °C for 1.5 h. Deproteinization was conducted with aq. NaOH (5%; w/v, 1:15) at 80 °C for 5 h. The product was then washed with distilled water until neutral reaction (pH 7), dried at 65 °C for 12 h, and decolorized in the Soxlet apparatus by refluxing a mixture of benzene and ethyl alcohol (v/v, 1:1) for 44 h. The isolated product was then dried at 65 °C for 22 h to yield pure Cn (28%). Elemental analysis, %: C, 46.33; N, 6.98; H, 6.36; ash, 0.08. The degree of acetylation (DA) of Cn (90.5%) was calculated by using elemental analysis data and an equation reported earlier (Kasaai, Arul, & Charlet, 2000).

2.3. Cs synthesis

Under nitrogen atmosphere, deacetylation of Cn (7 g) was carried out with aq. NaOH (50%; w/v, 1:14) and $NaBH_4$ (w/w, 10:1) at 117–120 °C for 2.5 h. The product was washed with distilled H_2O until neutral reaction (pH 7), then rinsed with ethyl alcohol (x3), acetone (x3), and dried in vacuum (10^{-1} torr; RT, 30 h) to yield Cs (5.7 g). Elemental analysis, %: C, 45.39; N, 7.70; H, 6.58; ash, 0.2. DA of synthesized Cs (47.6%) was calculated by using elemental analysis data and an equation reported earlier (Kasaai et al., 2000).

2.4. Cs purification

Cs (2.6 g) was dissolved in diluted acetic acid (0.19 M; 260 mL H_2O + 3 mL CH_3COOH) by continuously stirring the resulting suspension for 6.5 h. The mixture was then neutralized with NH_4OH (10%; 675 mL, 2.85 M; 1.5 h) and treated according to the literature protocol (Santos et al., 2005). The product was washed with distilled water until neutral reaction (pH 7) and dried in vacuum (10^{-1} torr; RT; 5.5 h) to yield Cs-pure (2.2 g).

2.5. Synthesis of aromatic aldehydes

4-(2-Chloroethyl)benzaldehyde (aldehyde-1) and 4-(2-bromoethyl)benzaldehyde (aldehyde-2) were synthesized by method described in Pogosyan and Matsoyan (1983).

A solution of 4-(2-chloroethyl) benzyl chloride (18.9 g, 0.1 mol) and hexamethylenetetramine (15.5 g, 0.11 mol) in dry chloroform (150 mL) was heated for 2 h at 70 °C. The reaction mixture was concentrated by 2/3 of the initial volume and diluted with ether (90 mL). The precipitate was filtered off, washed with ether, and refluxed in acetic acid (8 M; 90 mL) for 2 h. Hydrochloric acid was added (10 mL; 10 M) and the reaction mixture was refluxed for another 5 min. The crude mixture was extracted with ether, an organic layer was washed with water, then with aqueous sodium bicarbonate solution, then again with water and dried over $MgSO_4$. The reaction mixture was fractionated by vacuum distillation (103–105°/2 torr) to afford 4-(2-chloroethyl) benzaldehyde (aldehyde-1) (5.15 g, 31%), a colorless liquid. M.p. 32–33 °C. ^{1}H NMR (300 MHz, DMSO-d_6/CCl_4; 1/3, δ, ppm, *J*, Hz): 3.15 (2H, t, *J* = 7.0, CH_2), 3.80 (2H, t, *J* = 7.0, CH_2Cl); 7.43–7.48 (2H, m) and 7.80–7.85 (2H, m, C_6H_4); 9.96 (1H.s, CHO).

4-(2-Bromoethyl)benzaldehyde (aldehyde-2) was synthesized analogously (7.23 g, 49%) by using 4-(2-bromoethyl) benzylchloride as a reagent. A colorless liquid, m.p. 36–37 °C. ^{1}H NMR (300 MHz, DMSO-d_6/CCl_4; 1/3, δ, ppm, *J*, Hz): 3.15 (2H, t, *J* = 7.0, CH_2), 3.81 (2H, t, *J* = 7.4, CH_2Br), 7.43–7.48 (2H, m) and 7.80–7.85 (2H, m, C_6H_4), 9.96 (1H.s, CHO).

2.6. Synthesis of chitosan Schiff base (CsSB)

2.6.1. Cs-pure (Section 2.4) in the amount of 0.42 g (for the given DA of Cs of 47.6%, the amount of 2-amino-2-deoxy-D-glucopyranose units in the initial polysaccharide sample was 0.22 g, or 1.36 mmol) was stirred in a solution of H_2O (20 mL) and CH_3COOH (0.15 mL, 0.15 M) at RT for 7 h. A solution of aldehyde-1 (Section 2.5) (0.44 g; 2.61 mmol) in ethanol (5 mL) was added dropwise and the reaction mixture was stirred for additional 12 h. When adding aldehyde solution, the transparent and viscous (homogeneous) solution in the reactor gets cloudy, becomes heterogeneous, and light yellow residual matter gradually precipitates. The precipitate was filtered off, consecutively washed with acetone, ethanol and ether, and dried under reduced pressure (10^{-1} torr, RT, 4 h) to yield CsSBRTCl (0.46 g, 9.4%). The same synthesis was carried out at 80 °C by interaction of Cs-pure (Section 2.4) with aldehyde-1 (Section 2.5) to yield CsSB80Cl (0.45 g, 7.1%).

2.6.2. Cs-pure (0.83 g; Section 2.4; for the given DA of Cs of 47.6%, the amount of 2-amino-2-deoxy-D-glucopyranose units in the initial polysaccharide sample was 0.43 g, or 2.7 mmol) was dissolved in a mixture of H_2O (40 mL) and CH_3COOH (0.3 ml, 0.15 M) and stirred at RT for 7 h. A solution of aldehyde-2 (Section 2.5) (1.06 g, 5.0 mmol) in ethanol (10 mL) was added dropwise, and the reaction mixture was stirred for additional 12 h. When adding aldehyde solution, the transparent and viscous (homogeneous) solution in the reactor gets cloudy, becomes heterogeneous, and tan residual matter gradually precipitates. The precipitate was filtered off, consecutively washed with acetone, ethanol and ether, then dried