Unprecedented Chitosan-derived Selenoureas

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Abstract

Our research focuses on the synthesis of new selenoureidyl chitosan derivatives with tunable physicochemical properties by reaction of the biopolymer with a series of diversely substituted isoselenocyanates. All new compounds have been characterized by NMR techniques both in solution and in solid state. To study the applicability of the new chitosan-derived selenoureas, antioxidant capacity and GPx-mimics ability have been investigated.

Keywords

Chitosan; isoselenocyanates; selenoureidyl biopolymers.

Introduction

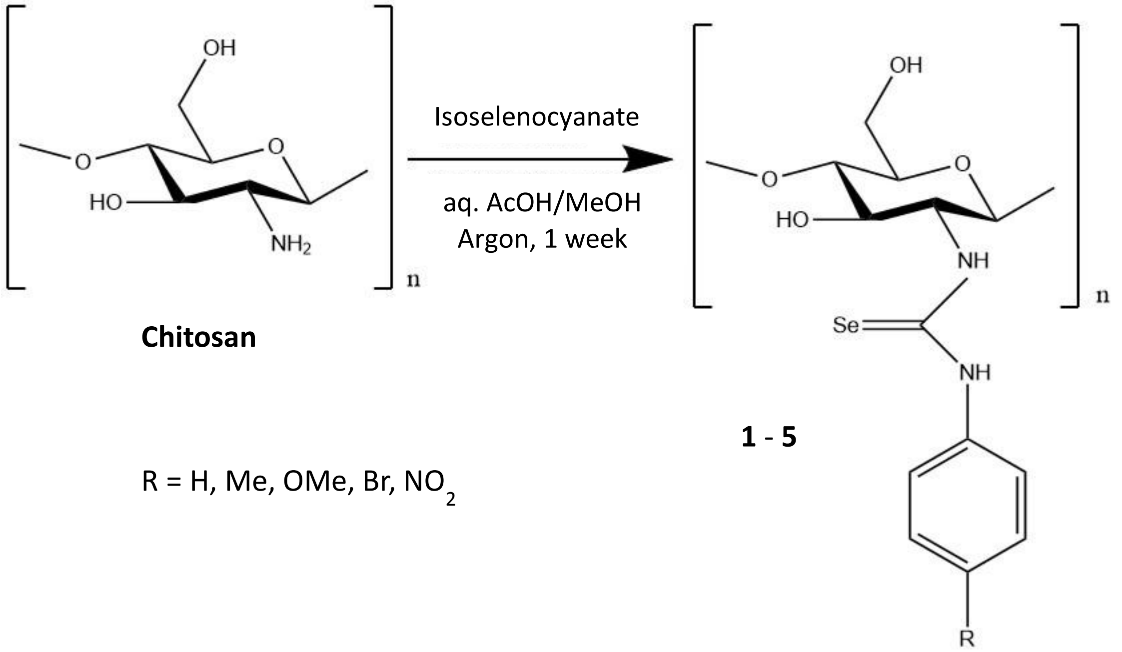
Chitosan, the chiral polysaccharide obtained by alkaline deacetylation of chitin has been applied in many fields, due to its biocompatible and biodegradable properties as well as its relative ease to manage compared to its chemical *N*-acetylated precursor.[1] The control of their chemical and physical properties allows chitosan derivatives to be used for different biomedical and industrial applications. Degree of deacetylation (DD) and polymer chain length (molecular weight, Mw) can be considered the most important features to determine the physico-chemical properties of these systems and consequently decisive factors to give desired results in formulations and applications.[2] For instance, chitosan can be used in several fields due to its sole properties such as drug delivery system, polar sensing biomaterial,[3] molecular NLO properties[4] or heterogeneous catalyst.[5]

Selenium is an essential trace element. In Nature, its main biological function is associated with the incorporation in the form of selenocysteine into certain proteins having redox motifs, such as Glutathione Peroxidase (GPx), among others. Different selenium containing compounds resulted to be bio-inspired catalysts, good antioxidant agents and good GPx-mimics.[6]

Herein, we report an easy synthesis of a variety of substituted aromatic isoselenocyanates with the Chitosan amino groups to obtain new Chitosan-derived selenoureas. All new compounds were characterized by means of ^{1}H, ^{13}C NMR both in solution and in solid state as well as mass spectrometry. Finally, antioxidant capacity and GPx-mimics ability for the synthesized selenoureidyl chitosan derivatives compounds have been investigated.

Results and discussion

Novel Chitosan-derived selenoureas (**1-5**) were synthesized in a heterogeneous mixture from corresponding isoselenocyanate and Chitosan (**Scheme 1**).



Scheme 1. Syntesis of Chitosan derived selenoureas (**1-5**)

Starting polymeric material was characterized to obtain the degree of deacetylation (DD = 84%) and molecular weight (Mw = 87875 g· mol^{-1}). Both parameters are the essential fingerprint for polymers characterization. For DD calculation, relative integrals of proton in ^{1}H-NMR spectra were used. Mw was determined by using GPC-SEC technique.

Isoselenocyanates were previously prepared using a described modified procedure[7] from the corresponding *p*-substituted phenyl formamides by treatment with triphosgene in dry CH_2Cl_2 containing Et_3N under reflux and subsequent treatment of the resulting isonitrile with black selenium in a one-pot processes to give the isoselenocyanate.

Characterization of Chitosan-derived selenoureas was realized by using FTIR, ^{1}H NMR and ^{13}C CPMAS NMR techniques. FRIR spectra for synthesized selenoureas show a band at 1646-1656 cm-1 (overlapped with C=O of amide moiety) for C=Se strtching vibration.

In ^{1}H NMR spectra for all obtained Chitosan derived selenoureas, the typical signals corresponding to Chitosan backbone were observed. When reaction between Chitosan and isoselenocyanates take place new peaks for aromatic protons were observed (see **Figure 1** as an example). Degree of *N*-substitution (DS) was calculated for each case from the ^{1}H NMR spectra by using de formula indicated in **Table 1.**

Table 1. Degrees of *N*-susbstitution calculated for Chitosan derived selenoureas

	Selenourea	**R**	***DS***
$\boldsymbol{DS\ (mol\%)} = \left[\frac{\boldsymbol{I(\frac{Ar}{n})}}{\boldsymbol{I(H2-H6)/6}}\right]\boldsymbol{x\ 100}$	**1**	H	32 %
	2	CH_3	25%
	3	OCH_3	27%
	4	Br	12%
	5	NO_2	1.4%

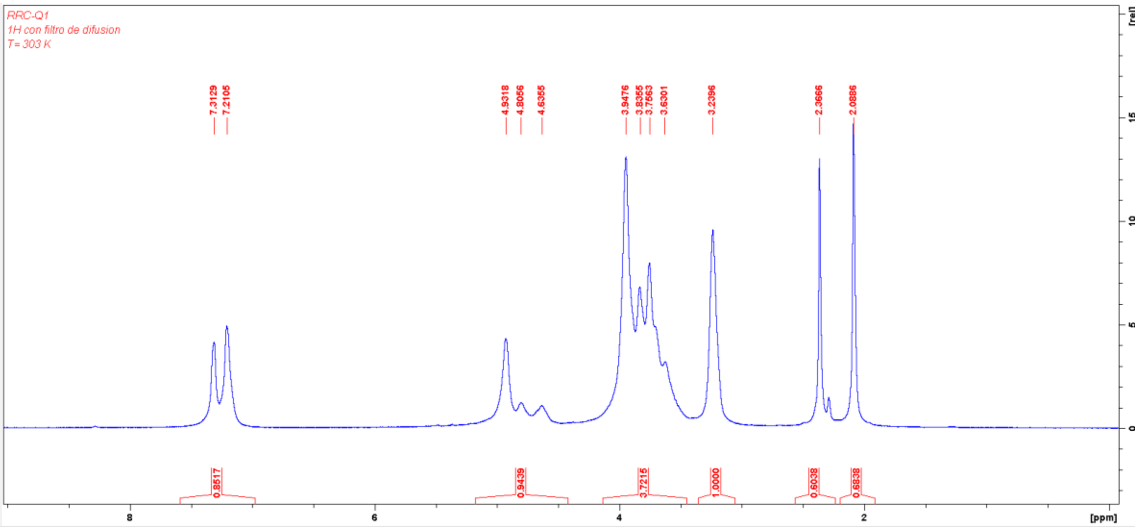


Figure 1. ^{1}H NMR of compound **2** in D_2O/AcOD at 150 MHz.

Additionally, ^{13}C CPMAS NMR spectra for all obtained Chitosan derived selenoureas, show next to the typical signals corresponding to Chitosan backbone the C=Se signal at 179-180 ppm (*e.g.* 179.5 ppm for compound **2**, see **Figure 2**) close to the C=O band of Chitosan amide moiety (173 ppm for compound **2**, **Figure 2**) and those corresponding to the aromatic carbons.

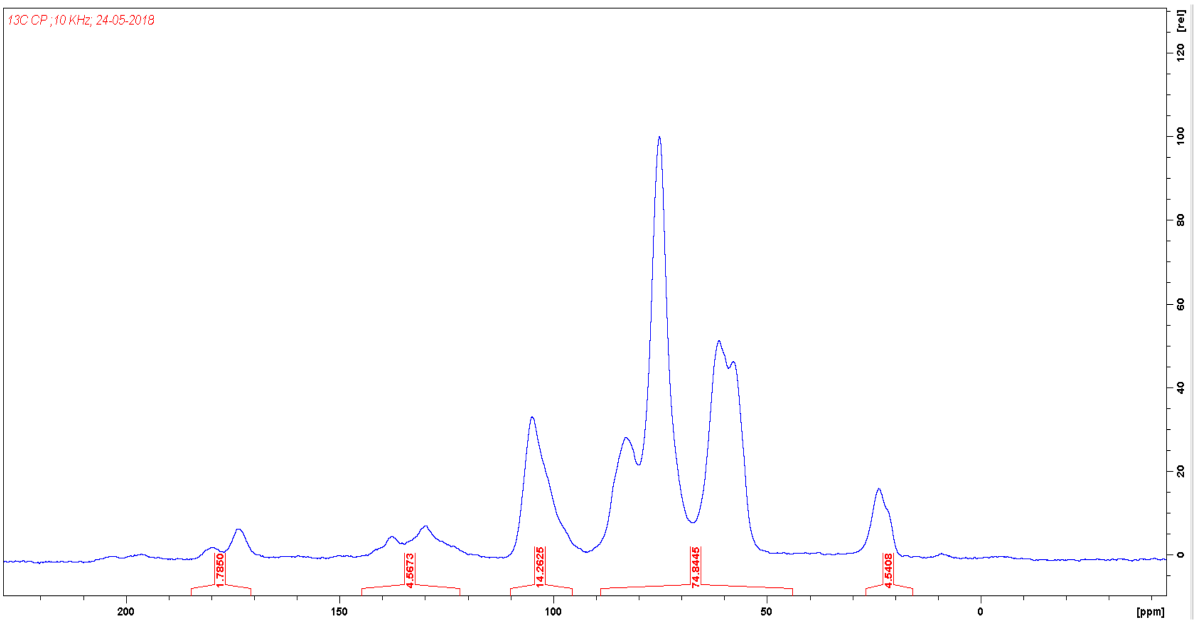


Figure 2. ^{13}C CPMAS NMR of compound **2** in solid state.

The antioxidant activity of new polymeric selenoureas **1-5** using the DPPH method as well the mimicry with the Glutathione peroxidase of these derivatives were evaluated, presenting the *p*-metoxyphenyl substituted compound **3** the best results (EC_{50} value of 0.402 ± ±0.001 µM and speed of reduction of H_2O_2 value vs. control of 1.13µM/s / 0.73µM/s, respectively).

Experimental Methods

All chemicals were purchased and used without further purification. Evaporations were conducted under reduced pressure. TLC was performed on silica gel plates (MN ALUGRAM Xtra SIL G/UV 254). All new compounds were synthesized following the below general procedure. Detection of compounds was accomplished with UV light (254 nm) and by charring with H_2SO_4 and characterization by ^{1}H and ^{13}C NMR, 13C CPMAS NMR spectroscopy and Mass spectrometry.

General procedure for the synthesis of selenoureidyl-Chitosan derivatives

To a heterogeneous stirred mixture of Chitosan (1 equiv.) in a 0.12M solution of aq. acetic acid in methanol at final pH value of 5, the corresponding aromatic isoselenocyanate (0.7 equiv.) was added under argon atmosphere at room temperature. The mixture was allowed to stand monitoring by TLC. After one week the mixture was filtered and the filtrate was washed successively to give the corresponding selenoureidyl-Chitosan derivative as a pink solid.

E.g. Spectral data for compound **2**:

IR ν_{max} 3230, 2890, 1656, 1542 cm^{-1}. ^{1}H RMN with filter difussion (500 MHz, AcDO, D_2O): δ 7.30 (d, J = 48.7, 4H), 4.91 (m, J = 149.6, 1H), 3.52 (m, J = 354.4, 6H), 2.32 (s, 3 H), 2.08 (s, 1H). ^{13}C CPMAS RMN (150.9 MHz): δ 179 (C=Se),173 (C=O, NAc), 150-120 (C1’-C6’), 105 (C1), 90-70 (C3-C6). 60 (C2), 20 (-CH_3).

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