New Coumarin-Chitosan based Fluorescent Polymers

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Abstract

Reductive amination of coumarin aldehydes with Chitosan as well as *N*-acylation of Chitosan owning different DD and Mw by a coumarin derived carboxylic acid have been carried out. Quaternization of the amino groups of the new chitosan derivatives that should provide water-soluble compounds has been also explored.

Keywords

Chitosan; Coumarins; Fluorescent biopolymers.

Introduction

Chitosan is a polysaccharide of β(1-4) linked D-glucosamine with a number of advantages such as non-toxicity, biocompatibility, and biodegradability.[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.

Fluorescent probes have a broad range of applications in biotechnology, biology, and health research and their use is continually increasing due to their versatility, sensitivity and quantitative capabilities. In particular, fluorescent polymers have application as fotoconductive resins, photosensitive materials, electroluminescent materials, getters photon, and as biological markers. On the other hand, trimethylated chitosan derivatives have emerged in recent years for gene delivery and engineering applications.[2] In this field, our specific interest is to develop pH probes for aberrant physiological changes.

We have previously reported the synthesis of a series of *N*-substituted chitosan derivatives from functionalized aromatic aldehydes, some of them showing fluorescence, others being hydrophobic molecules and others antimicrobial.[3] The syntheses were carried out by reductive amination of chitosan with substituted aromatic aldehydes and sodium cyanoborohydride as reducing agent.

In this communication, we have focused on the reductive amination of coumarin aldehydes with Chitosan as well as the *N*-acylation of Chitosan owning different DD and Mw by a carboxylic acid derived from coumarin. Additionally, quaternization of the amino groups of the new chitosan derivatives that should provide water-soluble compounds has been also explored.

Experimental Methods

All chemicals were purchased and used without further purification. Low molecular weight chitosan (Mw 87875 Da, PDI 1.81) with degree of deacetylation of 84% was purchased from Sigma-Aldrich. Chitosan of MW 69871 Da (PDI 2.39) with degree of deacetylation of 91% was prepared from Seefood Sevilla, S.L agro alimentary crabs shells' wastes (*Procambarus clarkii*). Evaporations were conducted under reduced pressure. TLC was performed on silica gel plates (DC-Alufolien F254, E. Merck); detection of compounds was accomplished with UV light (254 nm and 360 nm) and by charring with H_2SO_4 and characterization by HPLC-SEC, IR, NMR and Mass spectrometry.

*General Procedure for reductive amination of coumarin aldehydes. Synthesis of chitosan derivatives **1** and **2**.* To a solution of chitosan (0.42 mmol) in MeOH-water 1:1 at pH 5 (by using AcOH an NaOH 2M), a solution of the coumarin aldehyde **1a** or **2a** (0.4 mmol) in MeOH (30 mL) was added. The homogeneous mixture was stirred at room temperature for 24 h. Then, $NaBH_3CN$ was added in the case of **1a**, and $NaBH_4$ for **2a** and the mixture was stirred for 72 h. Product was precitated by treatment with NaOH 2M, pH 8-10, and the solid was washed with water (2x15 mL) and CH_2Cl_2 (2x15 mL) to obtain the corresponding chitosan derivatives **1** and **2**, after dried at reduced pressure. Incorporation degree (ID) for each case was determined by ^{1}H-NMR.

*Synthesis of coumarin-chitosan derivative **3**:* A solution of chitosan (0.12M AcOH) was added to a methanolic dispersion of coumarin carboxylic acid **3a** activated with DCC. Product was precipitated by addition of NaOH (1M) and filtered off. The solid was successively washed with H_2O, MeOH, EtOH and CH_2Cl_2 to obtain the coumarin-chitosan derivative **3**.

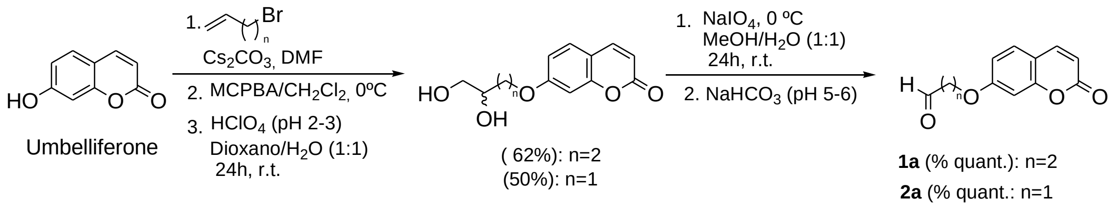
Quaternization of chitosan derivative ***3****:* Compound **3** was treated with NaI, NaOH 15% (w/v), MeI and *N*-metilpirrolidona at 60 ºC for 50 min. The product was precipitated with EtOH and then filtrated and treated once again with NaI, NaOH 15% (w/v), MeI and *N*-metilpirrolidona at 60 ºC for 30 min. The product was precipitated with EtOH, filtrated, washed with water and dried under reduced pressure.

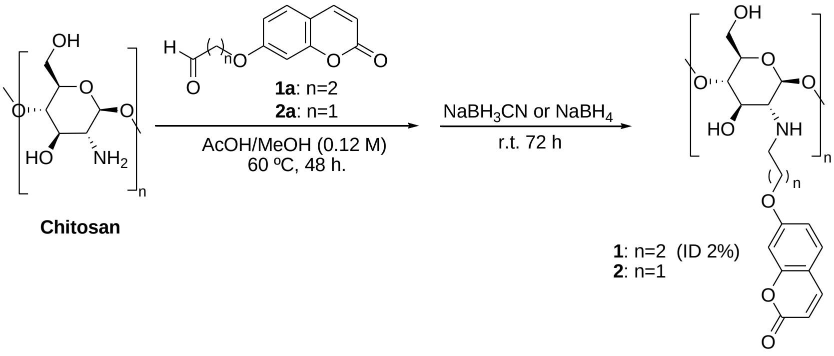
Chitosan derivatives were characterized by ^{1}H and ^{13}C NMR (500 and 125.7 MHz, respectively) in AcOD/D_2O as solvent and FTIR. For deacetylation degree calculation, freshly samples were prepared of chitosan in DCl/D_2O at 50ºC for 30 min. Fluorescence measurements were carried out using a Fluorescence Spectrophotometer Hitachi F2500.

Results and discussion

Multifunctional fluorescent derivatives of chitosan **1-3,** containing a coumarin chromophore unit, have been prepared. For joining the coumarin moiety to the polymer backbone we have developed two different strategies; each of them involves the formation of a different organic function. As a consequence, two different types of derivatives were obtained. The first type of derivatives, compounds **1** and **2**, containing a secondary amino function as the link, have been obtained by reductive amination of coumarin derived aldehydes with chitosan. The second strategy was *N*-acylation of chitosan amino groups with the appropriate carboxy coumarin compound.

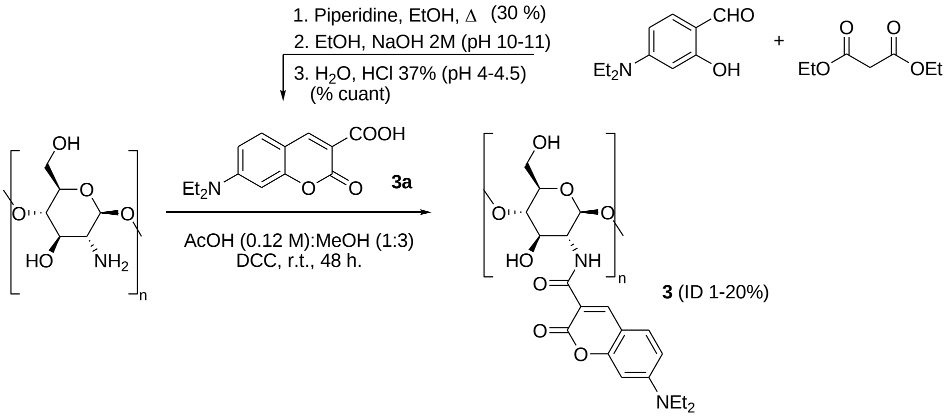
In Scheme 1 we present the synthesis of compounds **1** and **2**, by applying the first strategy. Previously, we carried out the synthesis of the corresponding coumarin precursor derivatives **1a** and **2a** starting from umbelliferone. In particular, compounds **1a** and **2a** were synthesized by oxidative cleavage of the diols,[4,5] in the dark with $NaIO_4$ as described in the literature.[6]





Scheme 1. Synthesis of coumarin-chitosan derivatives 1 and 2 by reductive amination.

Highly fluorescent compound **3a** was synthesized by Perkin reaction between 4-(diethylamino)salicylaldehyde and diethyl malonate in Piperidine/EtOH. Hydrolysis with NaOH or HCl gave compound **3a** in 30% yield after recrystallisation. [7] Activation of coumarinic acid **3a** with dicyclohexylcarbodiimide (DCC) and reaction with chitosan in a homogeneous or heterogeneus mixture gave compound **3** with a ID of 1 - 20% from commercial chitosan and an ID of 1% from chitosan obtained from crab wastes (Scheme 2).



Scheme 2. Synthesis of coumarin-chitosan derivative **1** obtained by a *N*-acylation reaction.

The new solvent- and pH-dependent fluorescent polymers have been characterized by HPLC-SEC, IR and NMR. For compound **3**, a Mw = 103006 Da (PDI 1.96) was determinate by HPLC-SEC. Representative fluorescence spectra obtained for some of new compounds, **1** and **3**, are showed in Figure 1.

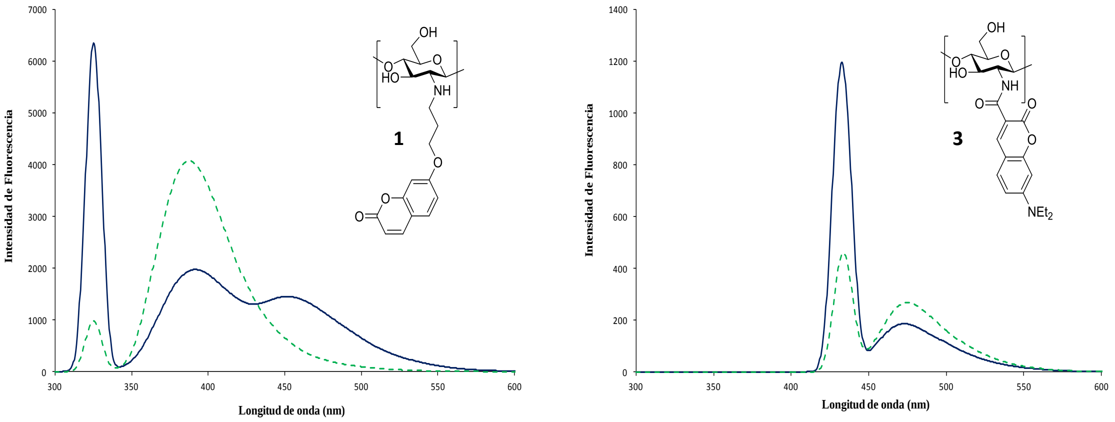


Figure 1. Fluorescence spectra for compounds **1** and **3** (——) and their corresponding precursors **1a** and **3a** (– –).

Quaternization of compound **3** was carried out in a two step process[8] and the structure of the resulting product is being investigated taking into account published data.[9]

Conclusions

In summary, we have successfully synthesized a variety of multifunctional fluorescent derivatives of chitosan, containing a coumarin chromophore unit, in an easy and cost-efficient way that provide a broad range of possible applications. Significant fluorescence of compound **3a** and **3** make interesting their use as fluorescent probes.

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REFERENCES

[1] Aranaz, I.; Harris, R.; Heras, A., *Curr. Org. Chem.* **2010**, *14*, 308-330.

[2] Raftery, R.; O'Brien, F. J., Cryan, S. A., *Molecules* **2013**, *18*, 5611-5647.

[3] S. Jatunov, A. Franconetti, M. Gómez-Guillén and F. Cabrera-Escribano; In *Proceedings of the 16th Int. Electron. Conf. Synth. Org. Chem., 1-30 November 2012*; Sciforum Electronic Conferences Series, 2012.

[4] Wahler, D.; Badalassi, F.; Crotti, P.; Reymond, J-L. *Chemistry – A European Journal* **2002**, *8*, 14.

[5] Zhu, Q.; Uttamchandani, M.; Li, D.; Lesaicherre, M.L.; Yao, S.Q. *Organic Letters* **2003**, *5*, 1257.

[6] Franconetti, A.; Borrachero, P.; Gómez-Guillén, M.; Cabrera-Escribano, F. *Synlett* **2013**, *24*, 0249

[7] Sanap, K.K.; Samant, S.D. *Synlett* **2012**, *23*, 2189.

[8] Martins, A.F.; Bueno, P.V.A.; Almeida, E.A.M.S. ; Rodrigues, F.H.A. ; Rubira A.F. ; Muniz, E.C. *Int. J. Biol. Macomol.* **2013**, *57*, 174.

[9] Sieval, A.B.; Thanou, M.; Kotzé, A.F.; Verhoef, J.C.; Brussee, J.; Junginger, H.E. *Carbohydr. Polymers* **1998**, *36*, 157.