

Synthesis of tailored Aromatic quaternized Chitosan probes as switchable redox devices

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Abstract

Involved in several physiological processes, redox behaviour has recently emerged as a promise research target requiring the design of compounds to be applied as sensor in a wide working window.

In this communication, the synthesis of new quaternized pyridinium-chitosan derivatives by reaction of the biopolymer with electronically tuneable 2,4,6-tri-arylpnyrylium tetrafluoroborates in mild conditions, and their characterization are described.

Keyword

Pyrylium complexes, Pyridinium salts, chitosan, fluorescence.

Introduction

Nowadays, redox sensors design is an important goal as a consequence of a possible wide range of applications. In this context, redox features have been exploited as metallic magnetic resonance contrast agent.¹ Furthermore, at cellular level a number of processes related to reactive oxidative species (ROS) and the maintenance of oxidative capability involve this kind of systems. In order to elucidate mitochondrial redox state, fluorescent sensor probes have been designed.²

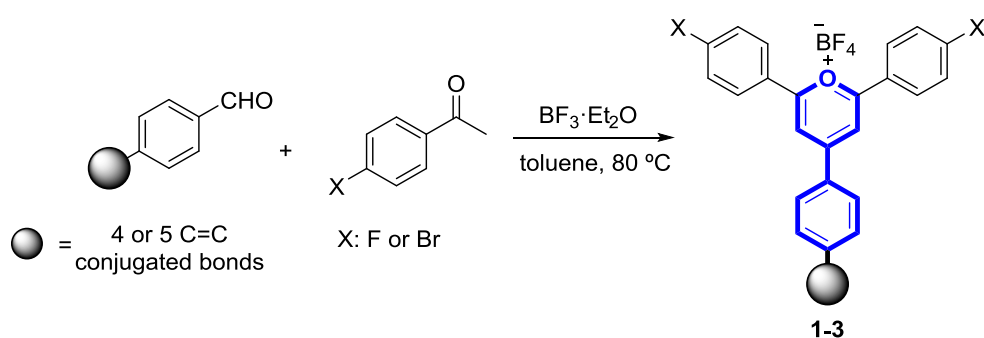
Pyridinium salts have been used as synthetic building blocks to obtain substituted pyridines, dihydropyridines or piperidines. In addition, pyridinium dyes present applications on multiple fields including biological and optical responses due to their photophysical features, in particular, high fluorescence, charge transfer character and solvatochromic properties. Recently, pyridinium-chitosan derivatives have shown NLO behaviour at molecular level as well as intrinsic fluorescent features.³

In order to obtain tailored pyridinium derivatives, a previous synthesis of the corresponding pyrylium salts which synthesis depends on the symmetry around the pyrylium cation is necessary. One of the available methods involves the direct reaction of 4-substituted benzaldehydes with 4-substituted acetophenones by using a Lewis acid catalyst which mediate cyclisation step.⁴ This kind of compounds constitutes a promising class of tunable emission wavelength dyes for laser technology applications.

Herein, we report the synthesis and characterization of new quaternized pyridinium-chitosan derivatives by reaction of the biopolymer with electronically tuneable 2,4,6-tri-arylpyrylium tetrafluoroborates in mild conditions to preserve the original physicochemical properties of the native chitosan. Fluorescence studies have been also performed on the polymeric biomaterials in order to demonstrate the redox sensor behaviour.

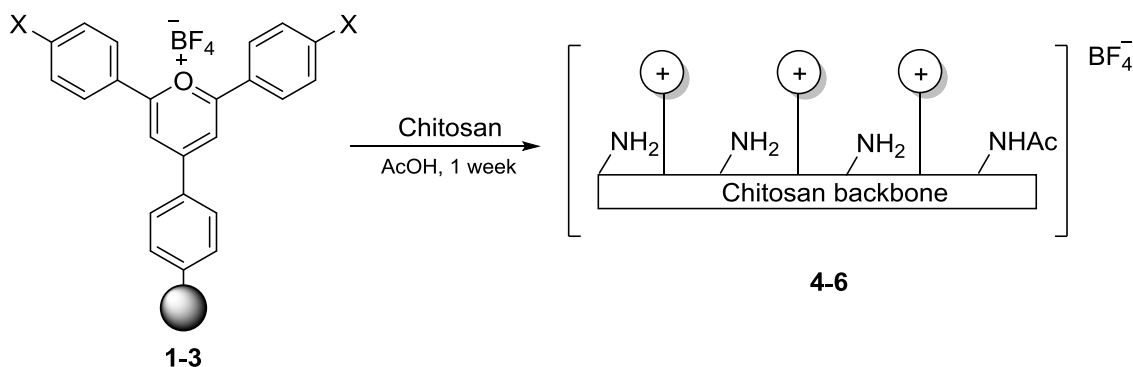
Results and discussion

Novel pyrylium salts (**1-3**) were synthesized with the aim to increase both conjugation pathway and emission wavelength (λ_e). $\text{BF}_3 \cdot \text{Et}_2\text{O}$ was used to mediate the cyclisation step of 1,5-dicarbonyl compound formed *in situ* in the reaction medium. It is known that fluorescent behaviour in solution is due to a the extended π -conjugation and substituent effect on position 2,6 have a minimum influence on absorption and fluorescence features.⁴ For this reason, fluorine and bromide have been chosen for a better synthesis procedure, exclusively (Scheme 1).



Scheme 1

Based on the reactivity of positions 2 and 6 which can be attacked by a nucleophile, amino groups of chitosan have been used to obtain pyridinium-chitosan derivatives (Scheme 2). Degrees of *N*-substitution (DS) proved to be pH-dependent. In this context, pH variations from 4 to 5.9 attaining a range of DS values have been performed.



Scheme 2

Chitosan derivatives have been characterized by using ^1H NMR experiments and ^{13}C CPMAS NMR in solid state. An effective attachment to chitosan backbone has been corroborated on the basis of diffusion NMR experiments.

When chitosan derivatives (**4-6**) was mixed with natural or synthetic reducing agents relative fluorescence intensity decays to zero as shown in Figure 1. This fact is presumable due to a loss of conjugation promoted by reduction of pyridinium cation to dehydropyridine moiety.

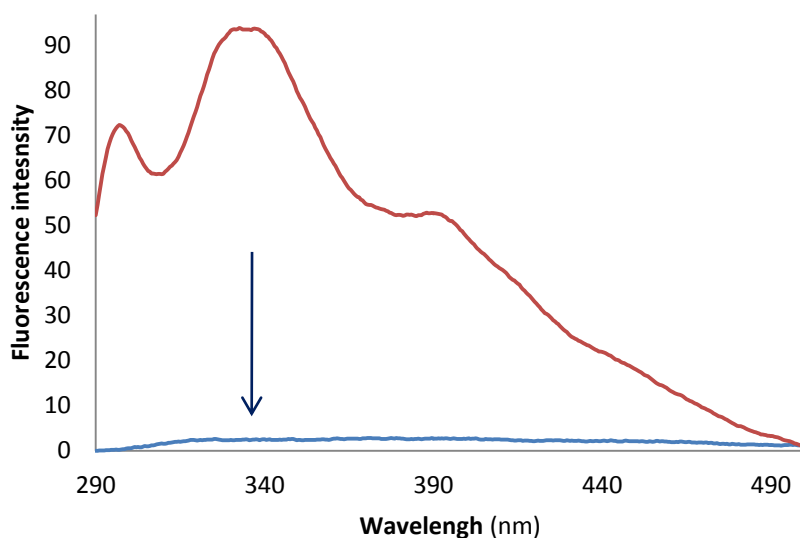


Figure 1

Conclusions

New pyrylium and pyridinium-chitosan derivatives have been synthesized in mild conditions in order to preserve the biopolymeric chain, and fully characterized. The main difference between other pyrylium salts was an increase of conjugation path and

an enhancement of fluorescent behaviour. Based on this feature, the use of these compounds as redox probes have been studied face to a several reducing agents.

Experimental Methods

All chemicals were purchased and used without further purification. Evaporations were conducted under reduced pressure. TLC was performed on silica gel plates (DC-Alufolien F₂₅₄, E. Merck). All new compounds were synthesized following the below general procedure. Detection of compounds was accomplished with UV light (254 nm) and by charring with H₂SO₄ and characterization by ¹H and ¹³C NMR spectroscopy and Mass spectrometry.

*General Procedure for synthesis of Pyrylium salts*⁵

BF₃·Et₂O (2.6 equiv.) was added dropwise under a N₂ atmosphere to a solution of *p*-substituted benzaldehyde (1 equiv.) and *p*-substituted acetophenone (2.5 equiv.) in toluene. The mixture was heated to 80 °C until the reaction was complete, monitored by TLC (1:4 EtOAc–hexane). The residue was treated with H₂O and EtOH. Crystallization from CH₂Cl₂ gave the corresponding pure pyrylium tetrafluoroborate.

General Procedure for synthesis of Pyridinium salts^{6,7}

To a solution of chitosan (100 mg) in aqueous acetic solution (25 mL), a solution of pyrylium tetrafluoroborate salt (0.30 mmol) in MeOH (100 mL) was added. The resulting solution was heated to 65 °C. The reaction mixture was left to reach the room temperature and then was treated with 4 M NaOH. The resulting solid was filtered off and successively washed with H₂O (2 x 2 mL), EtOH (2 x 4 mL), CH₂Cl₂ (2 x 2 mL) and acetone (2 x 2 mL) to give the corresponding pure chitosan-pyridinium salt.

Acknowledgements

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