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Title
Facile synthesis of acyl chitosan isothiocyanates and their application to porphyrin-appended chitosan derivative

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Abstract

Chitosan (1) was reacted with phenylisothiocyanate in 5% AcOH/ H2O to give N-phenylthiocarbamoyl chitosan (2) with a degree of substitution (DS) of N-phenylthiocarbamoyl groups of 0.86 in 87.1% yield. The following acylation of compound 2 with hexanoyl chloride in the presence of pyridine afforded 3,6-di-O-2,3-hexanoyl chitosan isothiocyanate (4a) with a DS of the isothiocyanate groups of 0.70 in high yield, unexpectedly. Compound 4a exhibited high levels of reactivity towards various amines to give the corresponding N-thiocarbamoyl chitosan derivatives in high yields. Other acyl (decanoyl (4b), myristroyl (4c), stearoyl (4d), benzoyl (4e)) chitosan isothiocyanates were also prepared from chitosan (1) in high yields. To evaluate the potential applications of acyl chitosan isothiocyanates, N-(triphenylporphynyl)thiocarbamoyl chitosan derivative 6 with a DS of the triphenylporphynyl groups of 0.46 was prepared from compound 4b. The Langmuir–Blodgett monolayer film of compound 6 gave a good photon-to-electron conversion performance.
Keywords

Acylation, Chitosan, Isothiocyanate, N-Phenylthiocarbamoylation, Photocurrent, Porphyrin
1. Introduction

Chitosan is a linear cationic heteropolymer of N-acetylglucosamine (GlcNAc) and glucosamine (GlcN) residues through β-1,4 linkages by the deacetylation of chitin which is the second most abundant natural biopolymer in nature, and a most versatile polysaccharide that lends itself to countless chemical and biochemical modifications (Muzzarelli, Tosi, Francescangeli & Muzzarelli 2003; Ravi Kumar et al. 2004; Muzzarelli R.A.A. & Muzarelli, C. 2005; Kurita 2006; Rinaudo 2006; Harish Prashanth & Tharanathan, 2007; Mourya & Inamdar, 2008; Sahoo D., Sahoo S., Morhanty, Sasmal & Nayak, 2009). However, considerable levels of attention have still been focused on the development of the high-value-added utilization for chitosan and its derivatives.

The N-substituted thiocarbamoyl chitosan derivatives which was prepared by N-thiocarbamoylation of chitosan with isothiocynate compounds are one of the important functional chitosan derivatives. For example, N-acetyl- (Ferkry & Mohamed 2010), N-acyl- (Zhong et al. 2008,), N-fluoresceinyl- (Qaqish & Amiji, 1999, Ma et al. 2008)), N-phenyl- (Baba, Noma, Nakayama & Matsushita, 2002, Monier & Abdel-Latif 2012) thiocarbamoyl chitosan derivatives has been reported as a corrosion inhibitor, an antimicrobial material, a macromolecular
fluorophore, a metal adsorbent, respectively. However, the availability of the commercial isothiocyanate compounds are limited. If chitosan isothiocyanate derivatives are easily synthesized, various amines are available for the syntheses of versatile \(N\)-substituted thiocarbamoyl chitosan derivatives for new applications. Glucosamine isothiocyanate derivatives can be prepared by the reaction of glucosamine with thiophosgene (Jochims & Seegler, 1965; Fernández-Bolaños, Zafra, López, Robina & Fuentes, 1999), but similar chitosan isothiocyanate derivatives have not been reported in the literature, even though chitosan has an amino group at its C-2 position that could be converted to an isothiocyanate group. The isothiocyanation of amines can be achieved by the reaction of an amine with thiophosgene or carbon disulfide (Mukerjee & Ashare, 1991; Fernández & Millet 1999, Munch, Hansen, Pittelkow, Christensen & Boas, 2008; Sun et al., 2012), although it is important to mention that both of these reagents are highly toxic. With this in mind, the development of a facile and safe synthetic method for the formation of chitosan isothiocyanate derivatives is strongly desired.

We recently reported a facile and safe synthetic method for acyl chitosan isothiocyanates by two reactions, that is, \(N\)-phenylthiocarbamoylation with
phenylisothiocyanate and acylation with acyl halide or acyl anhydride (Takano & Shibano, 2013). The resulting acyl chitosan isothiocyanates are soluble in common organic solvents and are expected to be useful synthetic intermediates for new functional chitosan derivatives. But, we did not report this procedure in its full detail.

On the other hand, the synthesis of porphyrin-containing chitosan derivatives represents one of several recent proposals for the high-value-added utilization of chitosan, with other examples including the construction of metallotetraphenylporphyrin appended chitosan derivatives (Huang, Guo & Tang, 2007), the use of an Mn (III) deuteroporphyrin-bearing chitosan as catalyst for oxidation reactions (Sun, Hu, Zhao & Liu, 2012), and tetraphenylporphyrin tethered chitosan derivatives for use as nanocarriers for gene delivery (Geware et al., 2013). The LB monolayer films of 6-O-porphynyl-2,3-di-O-stearoyl cellulose, which is a regioselectively substituted cellulose derivative, have been reported to exhibit high photon-to-electron conversion performances (Sakakibara, Ogawa & Nakatsubo, 2007). The high performance of this material has been attributed to the dense packing of the porphyrin moieties along the cellulose backbone because of the
well-defined and regular structure of the cellulose derivative. The
N-porphynylthiocarbamoyl chitosan derivatives prepared from the acyl chitosan
isothiocyanates could therefore potentially be used as alternative
photon-to-electron conversion materials.

This paper provides a detailed account of our new method for the synthesis
of acyl chitosan isothiocyanates (Scheme 1). Furthermore, we have described
the reactivity of these materials with various amines, and the preparation and
evaluation of an LB monolayer film of porphyrin-appended chitosan derivative
as one of the examples of the application of the isothiocyanates for the
preparation of functional chitosan derivatives.

2. Experimental

2.1. General

Chitosan (DAICHITOSAN 100D (VL), degree of deacetylation 98%) was kindly
supplied by Dainichiseika Color & Chemicals Manufacturing Co. (Tokyo, Japan).
All of the other chemicals used in the study were purchased from commercial
sources and used without further purification. Fourier-transform-infrared (FT-IR)
spectra were recorded on a Shimadzu IR Prestige-21 spectrophotometer
(Shimadzu, Kyoto, Japan) as KBr pellets (sample 1 mg/ KBr 200 mg). $^1$H and $^{13}$C NMR were recorded on a Varian 500 MHz FT-NMR spectrophotometer (Aglient Technologies, Santa Clara, CA, USA) using tetramethylsilane (TMS) as an internal reference standard in DMSO-$d_6$ or CDCl$_3$. The standard number of scans in the $^1$H and $^{13}$C NMR measurements were 3500 and 22000, respectively. The chemical shifts ($\delta$) of the NMR spectra have been reported in parts per million (ppm). UV–vis spectra were recorded on a Jasco V-560 UV–vis spectrophotometer (Jasco, Tokyo, Japan).

2.2. Preparation of acyl chitosan isothiocyanate

2.2.1. $N$-Phenylthiocarbamoylation

Chitosan (1, 1.20 g, 7.45 mmol) was dissolved in a 5% (v/v) solution of AcOH in water (30 mL) and the resulting solution was diluted with MeOH (120 mL). Phenyl isothiocyanate (5.34 ml, 44.7 mmol) was then added to the solution, and the resulting mixture was stirred at 35 °C for 24 h, during which time a precipitate formed. The precipitate was filtered, and the filter-cake was washed with MeOH before being collected and suspended in MeOH (300 mL) without drying. The suspension was then stirred at ambient temperature for 30 min and
filtered, and the filter-cake was washed with MeOH. This purification procedure was repeated several times until no absorbance could be detected at 280 nm in the filtrate. The solid product was then dried in vacuo to afford \( N \)-phenylthiocarbamoyl chitosan (2, 1.80 g, 87.1% yield).

**Compound 2 - DS \(_{PhNHCS} \):** 0.86 (determined by elemental analysis); FT-IR (KBr):

\[ \text{\nu} \text{ 3298, 2873, 1660, 1497, 1373, 1234, 1150, 1065, 898, 746, 692 \text{ cm}^{-1} \];

\[ \text{\( ^1 \)} \text{H NMR (DMSO-\( d_6 \)} : \delta 9.43 (NH), 7.80–7.00 (phenyl-H), 4.69 (H-1), 4.00–3.00 (H-2, H-3, H-4, H-5, H-6a, H-6b) ppm; \( ^{13} \text{C NMR (DMSO-\( d_6 \)} : \delta 182.0 (C=S), 139.5, 129.1, 124.5 \) (phenyl-C), 102.5 (C-1), 82.0 (C-4), 75.1 (C-5), 73.2 (C-3), 60.6 (C-6), 59.9 (C-2) ppm.

2.2.2. Acylation

**3,6-Di-O-hexanoyl chitosan isothiocyanate (4a) (typical method)**

Compound 2 (300 mg, 1.1 mmol) was suspended in a mixture of CHCl\(_3\) (6 mL) and pyridine (10 mL), and the resulting suspension was stirred at 35 °C for 24 h.

A solution of hexanoyl chloride (1.66 ml, 12.1 mmol) in CHCl\(_3\) (4 mL) was then added to the suspension in a drop-wise manner at 0 °C over a period of 10 min.

The resulting mixture was then stirred at 1–2 °C for 1 h before being heated at
30 °C for 1 h. The mixture was then heated at 80 °C for 18 h, before being cooled to ambient temperature and poured into MeOH (400 mL). The resulting mixture was stirred at ambient temperature for 2 h and formed a suspension, which was filtered. The filter-cake was then washed with MeOH before being collected and dissolved in a small amount of CHCl₃. The resulting solution was added to MeOH (400 mL) in a drop-wise manner to give a suspension, which was filtered. The filter-cake was then washed with MeOH before being collected and dried in vacuo to afford compound 4a (409 mg).

Compounds 4b-4e were also prepared according to the procedure for compound 4a. The DS, ¹H and ¹³C NMR and FT-IR data of compounds 4a-4e were summarized in Table 1.

2.3. Reactivity of hexanoyl chitosan isothiocyanate 4a with amines

3,6-Di-O-hexanoyl-N-phenylthiocarbamoyl chitosan (5a) (typical method)

Aniline (0.23 mL, 2.50 mmol) was added to a solution of compound 4a (200 mg) in THF (4 mL), and the resulting mixture was stirred at 35 °C for 24 h before being poured into distilled water (400 mL). The resulting precipitate was collected by filtration, and the filter-cake was washed with distilled water before
being collected and dissolved in a small amount of THF. The resulting solution was added to distilled water (400 mL) in a drop-wise manner to give a precipitate, which was collected by filtration. The filter-cake was then washed with distilled water before being collected and dried in vacuo at 40 °C to afford compound 5a (196 mg).

Compound 4a was also reacted with n-propyl amine and piperidine by the same procedure to give compounds 5b and 5c. The DS, ¹H and ¹³C NMR and FT-IR data of compounds 5a-5c were summarized in Table 1.

2.4. Application of decanoyl chitosan isothiocyanate (4b) to the formation of functional chitosan derivatives

2.4.1. Preparation of 3,6-di-O-heaxnoyl-N-(p-(10,15,20-triphenyl-5-porphyrinyl)phenyl thiocarbamoyl chitosan (6)

5-(4′-Aminophenyl)-10,15,20-triphenylporphyrin (TPP-NH₂) (29.1 mg), which was prepared according to the method reported by Luguya et al. (2004), was added to a solution of compound 4b (30 mg) in CH₂Cl₂ (4 mL), and the resulting mixture was stirred at 35 °C for 48 h in the absence of light before being poured
into MeOH (200 mL). The resulting precipitate was collected by centrifugation (3000 ×g, 15 min), and dissolved in a small amount of CH$_2$Cl$_2$. The resulting CH$_2$Cl$_2$ solution was then added to MeOH (200 mL) in a drop-wise manner to give a precipitate, which was collected by centrifugation (3000 ×g, 15 min). This precipitation/dissolution process was repeated three times. The solid product was then dried in vacuo at 40 °C to afford compound 6 (29 mg).

**Compound 6 - DSTPPNHCS**: 0.46 (determined by elemental analysis); FT-IR: ν

3415(NH), 2957, 2870, 2047, 1747 (C=O), 1537, 1498, 1377, 1356, 1242, 1167, 1107, 1053, 750, 696 cm$^{-1}$; $^1$H-NMR (CDCl$_3$): δ 8.80, 8.53, 8.18, 7.97, 7.38 (porphyrin-H), 5.40-3.10 (H-1, H-2, H-3, H-4, H-5, H-6a, H-6b), 2.36 (hexanoyl -OCOCH$_2$-), 1.60 (hexanoyl -OCOCH$_2$-CH$_2$-), 1.26 (hexanoyl -CH$_2$-), 0.88 (hexanoyl -CH$_3$), -2.80 (NH of porphyrin) ppm.

2.4.2. Preparation and evaluation of LB monolayer films of compound 6

A solution of compound 6 in CHCl$_3$ (0.5 mg/mL) was spread onto a water subphase in a Teflon-coated trough (331 × 100 × 5 mm, USI-3-22T, USI-system, Fukuoka, Japan). Ultrapure water was obtained from a Milli-Q water purification system (Simpli Lab, Merck Japan, Tokyo, Japan) and used for the subphase.
The solvent was evaporated for 30 min and the surface pressure ($\pi$)--area ($A$) isotherms were measured at a constant compression rate of 6 mm/min. The surface pressure was measured using a Wilhelmy-type film balance. The surface pressure was held at 10 mN m$^{-1}$ for 30 min prior to the deposition of the surface monolayer onto the substrates. The vertical dipping method was used to deposit the surface monolayer onto the substrate with quartz, or an Indium Tin Oxide (ITO) electrode. The downward and upward stroke rates were set at 6 mm/min. The surface pressure was held at 10 mN m$^{-1}$ throughout the deposition process, and the surface temperature was kept at 20 °C for the preparation of the LB monolayer films [i.e., film 6A (on quartz, transfer ratio: downward: 0.00, upward: 1.03), and film 6B (on an ITO electrode, transfer ratio: downward: 0.00, upward: 0.96)]. The photocurrent of film 6B was measured according to a previously reported method (Sakakibara, Ogawa & Nakatsubo, 2007).

3. Results and discussion

3.1. Preparation of acyl chitosan isothiocyanates

The $N$-phenylthiocarbamoylation of chitosan (1) was performed according to a
slightly modified version of the method reported by Baba et al. (2002). It is noteworthy that the authors of this particular study only reported part of FT-IR data during their characterization of the structure of \(N\)-phenylthiocarbamoyl chitosan (2). In terms of the \(N\)-phenylthiocarbamoylation of chitosan (1), chitosan was reacted with phenyl isothiocyanate in a mixture of 5% (v/v) AcOH in water and MeOH at 35 °C for 24 h to afford compound 2 in 87.1% yield. The FT-IR spectrum of this compound (Supporting information 1) contained characteristic bands derived from phenylthiocarbamoyl groups at 1541, 1497, 746, and 692 cm\(^{-1}\) (Monier & Abdel-Latif, 2012; Shibano, Kamitakahara & Takano, 2013). \(^1\)H and \(^{13}\)C NMR analyses of compound 2 revealed signals around 7.0 and 125–135 ppm, which were assigned to the aromatic protons and carbons of the phenylthiocarbamoyl group, respectively. The \(^{13}\)C NMR spectrum of compound 2 also contained a signal at 182.0 ppm, which was assigned to the C=S moiety of the phenylthiocarbamoyl group. The degree of substitution of the phenylthiocarbamoyl groups (DS\(_{\text{PhNHCS}}\)) in compound 2 was determined to be 0.86 by elemental analysis.

The hexanoylation of compound 2 was performed under typical acylation conditions (i.e., hexanoyl chloride and pyridine at 0 °C for 1 h, 30 °C for 1 h, and...
80 °C for 18 h sequentially) to give product A in high yield. Analysis of this compound by FT-IR revealed characteristic ester bands at 1747 and 1167 cm\(^{-1}\), whereas the band around 3298 cm\(^{-1}\) corresponding to the hydroxyl groups and NH moieties of the thioureido groups of compound 2 were absent. Signals characteristic of the hexanoyl groups (Zong, Kimura, Takahashi & Yamane, 2000) were also found in the \(^1\)H and \(^{13}\)C NMR spectra of product A (Fig.1). Taken together, these results suggested that hexanoylation had proceeded smoothly at both the O-3 and O-6 positions. In contrast, however, the characteristic bands of the phenylthiocarbamoyl groups at 1541, 1497, 746, and 692 cm\(^{-1}\) were not present in the FT-IR spectrum of product A. Furthermore, the aromatic signals of the phenyl moiety of the phenylthiocarbamoyl group around 7.0 and 125–135 ppm had disappeared from the \(^1\)H and \(^{13}\)C NMR spectra. These results therefore demonstrated, rather unexpectedly, that the phenylthiocarbamoyl groups were being removed from the chitosan during the hexanoylation process. The FT-IR spectrum of product A also contained a new band at 2047 cm\(^{-1}\), which was consistent with the introduction of isothiocyanate (i.e., -NCS) groups (Shibano, Kamitakahara & Takano, 2013). Furthermore, this band disappeared when product A was reacted with an amine, which provided
further evidence that this band related to the presence of NCS groups in product A. NMR analysis of provided further evidence in support of the presence of NCS groups in product A, with a signal consistent with the C=S moiety of the NCS group being observed at 140.8 ppm in the $^{13}$C NMR spectrum (Fig.1). Taken together, these data for product A indicated that this material was not 3,6-di-O-hexanoyl $N$-(hexanoyl)phenylthiocarbamoyl chitosan (3a) as expected, but 3,6-di-O-hexanoyl chitosan isothiocyanate (4a). The DS$_{NCS}$ of compound 4a was determined to be 0.74 by elemental analysis.

Fig.2 shows the FT-IR spectra of the products resulting from the hexanoylation of compound 2 at various time points during the 80 °C heating stage of the reaction. The results of this analysis revealed that the characteristic bands of the ester and amide groups at 1747 and 1167 cm$^{-1}$ and 1678 cm$^{-1}$, respectively, (Mohamed & Abd El-Ghany, 2012) appeared rapidly after only 1 h, whereas the bands attributed to the hydroxyl and thiourea groups at 3298 cm$^{-1}$ were reduced significantly. These changes in the FT-IR spectra indicated that the O-hexanoylation of the 3-OH and 6-OH positions had proceeded smoothly, as well as the N-hexanoylation of the phenylthiocarbamoyl groups. The $^1$H NMR spectrum of the product after 1 h, however, showed that the
O-hexanoylation process had not proceeded to completion (data not shown).

The FT-IR spectrum of the product after 1 h of the 80 °C heating stage contained a small band at 2047 cm$^{-1}$ for the NCS groups, which suggested that the N-phenylthiocarbamoyl groups were beginning to degrade during the first hour of this heating stage. As the reaction increased, there was an increase in the intensity of the band at 2047 cm$^{-1}$, whereas the intensities of the bands at 1678, 1541, 1497, 746, and 692 cm$^{-1}$ decreased. After 18 h, the bands at 1678, 1541, 1497, 746, and 692 cm$^{-1}$ were disappeared completely, suggesting that the N-(hexanoyl)phenylthiocarbamoyl groups had been fully degraded.

N,N'-Disubstituted thioureas are known to decompose to the corresponding amines and isothiocyanates when they are heated (Mukerjee & Ashare, 1991). For example, the pyrolysis of N-benzoyl-N'-phenylthiourea at 180 °C was reported to afford phenyl isothiocyanate in high yield (Rajappa, Rajagopalan, Sreenivasan & Kanal, 1979). Based on these reports and the FT-IR spectra shown in Fig.2, we have proposed a mechanism for this transformation which is shown in Fig.3. Briefly, the phenylthiocarbamoyl groups of compound 2 would be converted to the N,N-(hexanoyl)phenylthiocarbamoyl groups during O-hexanoylation process. The
N,N-(hexanoyl)phenylthiocarbamoyl groups would then be degraded by the abstraction of a proton by pyridine, which would resulted in the formation of the NCS groups.

To evaluate the versatility of this method, we investigated the use of several other acylating agents for the acylation of compound 2 (i.e., dodecanoylation, myristoylation, stearoylation, and benzylation) under the same conditions as those used for the hexanoylation reaction, which afforded compounds 4b–e in high yields. The FT-IR spectra of compounds 4a–d revealed that the characteristic bands of the phenylthiocarbamoyl groups at 1541, 1497, 746, and 694 cm\(^{-1}\) had disappeared, and that the characteristic bands of the NCS and ester groups had appeared around 2047 cm\(^{-1}\), and around 1748 and 1159 cm\(^{-1}\), respectively (Supporting information 1). These results indicated that the isothiocyanation reaction had proceeded in all cases regardless of the acyl group used in the acylation reaction. The DS\(_{NCS}\) values of compounds 4b–d and 4e were determined to be 0.70 and 0.56, respectively, by elemental analysis. The solubility of compound 2, as well as those of compounds 4a–e are summarized in Table 2. The acyl chitosan isothiocyanates 4a–e were found to be soluble in a range of common solvents, including THF,
CHCl₃, and CH₂Cl₂. Interestingly, however, compounds 4a–e became insoluble in these solvents when they were stored as drying solids at ambient temperature for more than several days. Subsequent testing of the insoluble solid materials by FT-IR spectroscopy revealed that they were analytically identical to the initial solids (data not shown). Similar insolubilization behavior has also been observed for compound 2 and 6-isothiocyanato cellulose derivatives (Shibano, Kamitakahara & Takano, 2013).

The N-phenylthiocarbamoylation of chitosan with phenyl isothiocyanate, followed by acylation with acyl chloride under basic conditions (i.e., in the presence of pyridine) has therefore been demonstrated as effective process for the preparation of acyl chitosan isothiocyanates. Furthermore, this method allows for the use of harmful reagents such as thiophosgene to be avoided. In many ways, our newly developed method represents a trans-isothiocyanation reaction from a phenyl isothiocyanate to an acyl chitosan isothiocyanates in two reactions.

3.2. Reactivity of hexanoyl chitosan isothiocyanate 4a with amines

Sugar isothiocyanates are known to react readily with amines to form thioureas
(Pérez, Mellet, Fuentes & Fernández, 2000). To confirm it, we proceeded to investigate the reactivity of the acyl chitosan isothiocyanates towards a variety of amines. When compound 4a was reacted with aniline (aromatic amine) in THF at 35 °C for 24 h, compound 5a was formed in high yield. The FT-IR spectrum of compound 5a contained the characteristic bands of the phenylthiocarbamoyl groups at 1537, 1497, 750, and 696 cm\(^{-1}\), whereas the characteristic NCS band at 2047 cm\(^{-1}\) had disappeared. Furthermore, the \(^{13}\)C NMR spectrum of compound 5a contained a new signal at 181.0 ppm for the C=S moiety of the newly formed phenylthiocarbamoyl group, which indicated that the reaction of compound 4a with aniline had proceeded smoothly. Compound 4a was also reacted with propyl amine (aliphatic primary amine) and piperidine (aliphatic secondary amine) under the same conditions to give the corresponding compounds 5b and 5c in high yields, respectively. These results demonstrated that the acyl chitosan isothiocyanates were highly reactive towards amino compounds, and could therefore be used as intermediates for the synthesis of N-thiocarbamoyl chitosan derivatives.

3.3. *Formation of a functional chitosan derivative from decanoyl chitosan*
isothiocyanate 4b

The acyl chitosan isothiocyanate 4b was converted to the porphyrin-appended chitosan derivative 6 to demonstrate the potential application of these compounds for the formation of functional chitosan derivatives. Compound 4b was reacted with TPP-NH₂ in CH₂Cl₂ at 35 °C for 48 h to give compounds 6 in high yield. The FT-IR spectrum of compound 6 contained the characteristic bands of decanoyl chitosan at 2926, 2854, 1744, 1155, 1111, and 1055 cm⁻¹, as well as those from the porphyrin at 3415, 1597, 1468, 1350, 1178, 966, 800, 732, and 702 cm⁻¹, and those from the thiourea groups at 1547 cm⁻¹ (Fig. 4). It is noteworthy that a small band corresponding to the NCS group was detected at 2039 cm⁻¹ in FT-IR spectrum of compound 6, which indicated that the reaction with TPP-NH₂ had not proceeded to completion. The ¹H NMR spectrum of compound 6 contained signals from the aromatic protons of the porphyrin ring in the range 7.2–9.0 ppm, as well as the pyrrole-NH proton of the porphyrin ring at –2.80 ppm (Luguya et al. 2004) (Supporting information 2). The UV-vis spectrum of compound 6 in chloroform contained a Soret band in the range of 350–450 nm (Supporting information 3). These results clearly indicated that compound 6 was the expected porphyrin-appended chitosan
derivative. The $\text{DS}_{\text{TPPNHCS}}$ value of compound 6 was determined to be 0.46 by elemental analysis. This medium DS value was attributed to the steric hindrance of the porphyrin groups, because a similar effect was also observed in the corresponding porphyrin-appended cellulose derivative (Sakakibara, Ogawa & Nakatsubo, 2007).

LB monolayer films of compound 6 were prepared on quartz (film 6A) and on an ITO electrode (film 6B) using the vertical dipping method with surface a pressure of 5 mN/m, which was decided based on the surface pressure ($\pi$)-area ($A$) isotherm of compound 6 at the air-water interface at 20 °C (Supporting information 4). In both cases, the monolayer film on the water was not transferred during the first down stroke, but was transferred during the second up stroke with a transfer ratio of almost 1.0, which indicated that films 6A and 6B were Z-type LB films. Film 6A was subjected to UV-vis analysis, whereas 6B was evaluated in terms of its photocurrent generation performance. The UV-vis spectrum of film 6A (solid state) had a similar profile to that of compound 6 in chloroform (solution state), which suggested that the monolayer had been successfully transferred. Fig. 5i shows the photoelectrochemical response of film 6B with illumination at 420 nm. The photocurrent was
generated quickly when film 6B was illuminated. Fig. 5ii shows the action
spectrum of film 6B (circles) and the UV-vis spectrum of film 6A (solid line). The
patterns of these two spectra were very similar, which suggested that the
porphyrin moieties of compound 6 were effectively behaving as photoactive
species for the generation of the photocurrent, based on the absorption
spectrum. The photocurrent density (i.e., photocurrent per unit area of a
working electrode) for film 6B at 420 nm was 236 μA/cm². This value was lower
than that of an LB monolayer film constructed from a porphyrin-appended
cellulose derivative, which had a DS_{porphyrine} value of 0.64 (Sakakibara, Ogawa
& Nakatsubo, 2007), and could therefore have been lower because of the lower
DS_{TPPNH2} value of compound 6. Taken together, these results suggest that
compound 6 could be used as an effective alternative photon-to-electron
conversion material in biomaterial-based solar cells.

4. Conclusion

A facile new method has been developed for the synthesis of for the preparation
of acyl chitosan isothiocyanates based on the N-phenylthiocarbamoylation of
chitosan followed by acylation of the resulting thiocarbamoylated material under
basic conditions. Surprisingly, the formation of the NCS groups of the acyl chitosan isothiocyanates occurred as a consequence of the degradation of the N,N-(acyl)phenylthiocarbamoyl groups under the basic conditions required of the acylation reaction. A similar outcome was observed when the acylation reaction was conducted with acyl anhydride species under basic conditions, and the details of this alternative method will be published in our next paper.

The acyl chitosan isothiocyanates exhibited a high level of reactivity towards amines to afford the corresponding N-thiocarbamoyl chitosan derivatives, which suggested that various functional amines could be used to for the functionalization of chitosan. A porphyrin-appended chitosan derivative (6) was also prepared to evaluate the application of these acyl chitosan isothiocyanates to the synthesis of functional materials. The LB monolayer film of compound 6 gave a good photon-to-electron conversion performance, which suggested that compound 6 could be used as a promising photon-to-electron conversion material. Taken together, the results of this study demonstrate that our new method can be used to be provide rapid access to a range of acyl chitosan isothiocyanates, which have the potential to become useful intermediates for the construction of functional chitosan derivatives.
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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.106/j.carbpol.

References


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(Figures & Table)

**Scheme 1** Preparation of N-substituted thiocarbamoyl chitosan derivatives (5a–c and 6) via the corresponding acyl chitosan isothiocyanates (4a–e).

**Figure 1.** $^1$H and $^{13}$C NMR spectra of product A (Compound 4a).

**Figure 2.** FT-IR spectra of the products during the 80 °C heating stage for the hexanoylation of compound 2 (normalized at 1379 cm$^{-1}$).

**Figure 3.** Proposed reaction mechanism for the formation of isothiocyanate groups.

**Figure 4.** FT-IR spectra of compounds 4b (A); 6 (B); and TPP-NH$_2$ (C).

**Figure 5.** (i) Photoelectrochemical response of the LB monolayer film 6B with illumination at 420 nm; (ii) Action spectrum of film 6B (circles); UV-vis spectrum of film 6A (solid line).

**Table 1.** Data of compounds 4a–e and 5a–c

**Table 2.** Solubility of chitosan derivatives 2 and 4a–e

(Supporting information)

**Supporting information 1**
FT-IR spectra of compounds 1, 2, 4a–e and 5a–c.

**Supporting information 2**
$^1$H NMR spectrum of compound 6.

**Supporting information 3**
UV-vis spectra of compound 6 in CHCl$_3$ (solid line) and the LB monolayer film 6A (dashed line) (normalized at 424 nm).
Supporting information 4

Surface pressure ($\pi$)-area ($A$) isotherm of compound 6
Scheme 1 Preparation of $N$-substituted thiocarbamoyl chitosan derivatives (5a-c and 6) via acyl chitosan isothiocyanates (4a-e)
Figure 1. $^1$H- and $^{13}$C-NMR spectra of product A (Compound 4a)
Figure 2. FT-IR spectra of the products at the 80°C stage in hexanoylation of compound 2 (normalized at 1379 cm⁻¹)
**Figure 3.** Proposed reaction mechanism for the formation of isothiocyanate groups
Figure 4. FT-IR spectra of compounds 4b (A); 6 (B); TPP-NH₂ (C)
Figure 5. (i) Photoelectrochemical response of the LB monolayer film 6B with illumination at 420 nm; (ii) Action spectrum of film 6B (circles); UV-vis spectrum of film 6A (solid line).
Table 1 Data of compounds 4a-e and 5a-c

<table>
<thead>
<tr>
<th>Compound (Acyl group)</th>
<th>4a (hexanoyl)</th>
<th>4b (decanoyl)</th>
<th>4c (myristroyl)</th>
<th>4d (stearoyl)</th>
<th>4e (benzoyl)</th>
<th>5a (hexanoyl)</th>
<th>5b (hexanoyl)</th>
<th>5c (hexanoyl)</th>
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<tr>
<td>DS*</td>
<td>0.74</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
<td>0.56</td>
<td>0.68</td>
<td>0.68</td>
<td>0.64</td>
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<td>NCS</td>
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<td>NCS</td>
<td>NCS</td>
<td>NCS</td>
<td>NCS</td>
<td>PhNHCS-</td>
<td>PrNHCS-</td>
<td>PiperidylNHCS-</td>
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<td>H-3</td>
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<td>5.17</td>
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<td>5.03</td>
<td>5.10</td>
<td>5.01</td>
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<td>4.00-3.40</td>
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<td>H-2, H-4, H-5</td>
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<td>3.67</td>
<td>3.67</td>
<td>3.67</td>
<td>4.00-3.40</td>
<td>4.00-3.40</td>
<td>4.00-3.40</td>
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<tr>
<td>acyl -OCOCH2-</td>
<td>2.37</td>
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<td>-</td>
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<td>-</td>
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<tr>
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<tr>
<td>Others</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>7.60-7.06</td>
<td>2.34, 1.32, 0.89</td>
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1H NMR (in CDCl3) (ppm)

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<th>13C NMR (in CDCl3) (ppm)</th>
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<tbody>
<tr>
<td>C=S</td>
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<td>C=O</td>
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<tr>
<td>NCS</td>
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<tr>
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<td>C-2</td>
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<tr>
<td>C-3</td>
</tr>
<tr>
<td>C-4</td>
</tr>
<tr>
<td>C-5</td>
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<tr>
<td>acyl -C</td>
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<tr>
<td>FT-IR (cm⁻¹)</td>
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* The DS (degree of substitution) were determined by elementary analyses.
Table 2: Solubility of chitosan derivatives 2 and 4a-4e

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<tr>
<th>Solvents</th>
<th>δ</th>
<th>2</th>
<th>4a</th>
<th>4b</th>
<th>4c</th>
<th>4d</th>
<th>4e</th>
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<td>THF</td>
<td>9.1</td>
<td>×</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Chloroform</td>
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<td>×</td>
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<td>○</td>
<td>○</td>
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<tr>
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<td>×</td>
<td>○</td>
<td>△</td>
<td>△</td>
<td>×</td>
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<tr>
<td>Dichloromethane</td>
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<td>×</td>
<td>○</td>
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<tr>
<td>Dioxane</td>
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<td>×</td>
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<td>○</td>
<td>△</td>
<td>△</td>
<td>×</td>
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<tr>
<td>DMF</td>
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<td>○</td>
<td>○</td>
<td>△</td>
<td>△</td>
<td>×</td>
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<tr>
<td>DMSO</td>
<td>12.8</td>
<td>○</td>
<td>△</td>
<td>△</td>
<td>×</td>
<td>×</td>
<td>○</td>
</tr>
<tr>
<td>Methanol</td>
<td>12.9</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
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<td>×</td>
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<tr>
<td>Water</td>
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<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
</tbody>
</table>

δ: Solubility parameter; ○: Soluble, △: Partially soluble, ×: Insoluble
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$^1$H-NMR spectrum of compound 6.
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