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Note

Preparation of mixed 10-undecenoyl/phenylaminocarbonyl or benzoyl derivatives of chitosan

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Abstract

The preparation of mixed 10-undecenoyl/phenylaminocarbonyl or benzoyl derivatives of chitosan is described. Several mixed derivatives were prepared using differently substituted aryl isocyanates or benzoyl chlorides. The reactivity of the starting polysaccharide was found to be influenced by the acetyl content. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Chitosan; Chitin; Polysaccharides; Degree of substitution

1. Introduction

One of the fields in which polysaccharides have been developed is for chiral chromatography. Polysaccharides and their derivatives, being chiral, show chiral recognition for enantiomers. This feature has been exploited in the preparation of chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC). These CSPs usually consist of a polysaccharide-derived selector coated onto a chromatographic matrix. Several derivatives of cellulose and amylose have been developed for this purpose [1]. Such derivatives are prepared by the reaction of the corresponding polysaccharide with aryl isocyanates or benzoyl chlorides to obtain carbamates or esters, respectively. The presence of aromatic groups attached to the polysaccharide structure increases the chiral recognition ability of these compounds.

Nevertheless, once the polysaccharides have been chemically modified by introducing hydrophobic groups, the resulting derivatives become soluble in many organic solvents. This represents a serious drawback in chromatography, since the range of solvents that can be used in the mobile phase is limited when coating-based CSPs are involved. Some attempts to bond the polysaccharide-derived selector chemically have been described. One of them consists of the preparation of mixed deriva-

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tives in which 10-undecenoyl groups are introduced on the polysaccharide molecule together with the aromatic groups [2–5]. The method has also been applied to chitosan derivatives, whose chromatographic behavior has recently been described [6].

The aim of this study was to describe a reproducible procedure to obtain mixed 10-undecenoyl/aryl derivatives of chitosan with a defined ratio of substituents. Several mixed derivatives were prepared and characterized.

2. Results and discussion

Previous treatment of commercial chitosan.—Chitosan is obtained from chitin, a (1→4)-2-acetamido-2-deoxy-β-D-glucan, by alkaline hydrolysis. It is usually commercialized as off-white platelets or flakes. In a first attempt, this commercially available material was submitted to the same reaction conditions as cellulose [4,5]. However, an almost unreacted chitosan mixture was given back even when a large excess of reagent was used. This low reactivity was attributed to the difficulty in dispersing this material in the reaction medium (pyridine). In order to enhance the reactivity, this commercial chitosan was dissolved and reprecipitated to improve its dispersion in solvents [7]. The resulting product was a white fine powder that was characterized spectroscopically and by elemental analysis before derivatization. Two batches of chitosan were used in this study (**A** and **B**). The acetyl content of the reprecipitated chitosan **A** was estimated to be ≈20%, whereas that of **B** was ~13%, according to the ^{1}H NMR and elemental analysis [8].

Preparation of mixed 10-undecenoyl/aryl derivatives of chitosan.—Chitosan **B** was chosen for optimization of the derivatization process. According to previous studies [9], chitosan having derivatives with bulky *N*-aryl groups enhances the accessibility of the hydroxyl groups. Hence, the prior introduction of such aromatic groups may facilitate the introduction of the alkenoyl chains later (Fig. 1).

Chitosan and 3,5-dimethylphenyl isocyanate were allowed to react in pyridine under various reaction conditions (reaction time, temperature). The highest DS was obtained at 115 °C within a reasonable time (24 h), when an excess of 6 mol of isocyanate per glucosamine unit was used, and a DS of 2.1 was attained. After 30 min of reaction at reflux temperature, the mixture changed its appearance and became viscous. That point was considered the optimal moment for adding the second reagent, 10-undecenoyl chloride, in the preparation of mixed derivatives.

In subsequent experiments, once the expected viscous solution was obtained, 0.5 mol of 10-undecenoyl chloride per mol of glucosamine residues was added, and the mixture

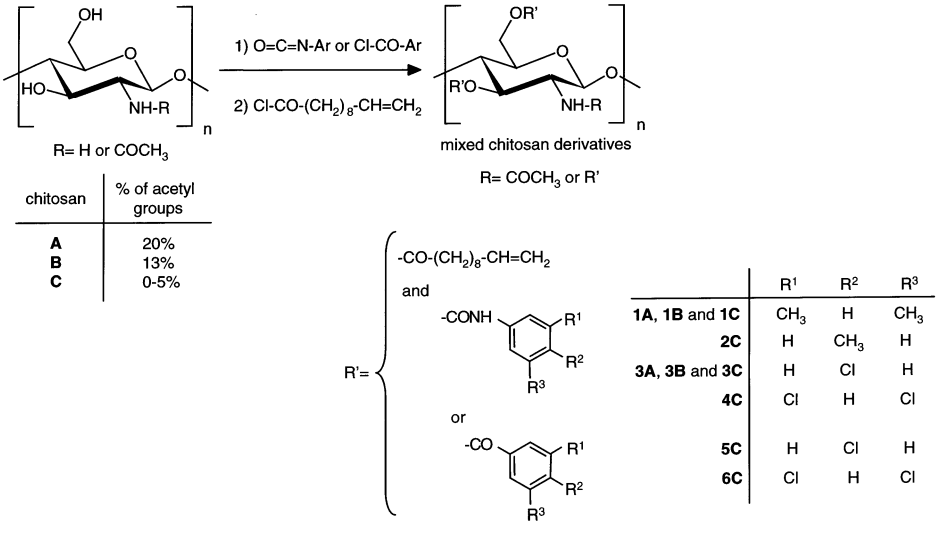

Fig. 1. Preparation of mixed 10-undecenoyl/phenylaminocarbonyl and benzoyl derivatives of chitosan.

Table 1
Elemental analyses of 10-undecenoyl/arylderivatives of chitosan

Aryl derivative	Sample	% Acetyl groups	Found [a]				Degree of substitution [b]		Calculated			
			%C	%H	%N	%Cl	10-Undec	Ar	%C	%H	%N	%Cl
3,5-Dimethylphenylaminocarbonyl	**1A**	20	58.07	6.31	7.98	–	0.13 ± 0.06	1.38 ± 0.38	61.61	6.65	8.44	–
3,5-Dimethylphenylaminocarbonyl	**1B**	13	60.04	6.30	8.09	–	0.16 ± 0.07	1.87 ± 0.57	63.73	6.64	8.55	–
3,5-Dimethylphenylaminocarbonyl	**1C**	0–5	61.69	6.32	8.06	–	0.24 ± 0.08	2.22 ± 0.56	65.14	6.69	8.52	–
4-Methylphenylaminocarbonyl	**2C**	0–5	61.30	5.92	8.69	–	0.25 ± 0.07	2.31 ± 0.38	64.09	6.19	9.09	–
4-Chlorophenylaminocarbonyl	**3A**	20	52.80	4.96	7.38	13.12	0.39 ± 0.10	1.66 ± 0.31	54.69	5.13	7.63	12.01
4-Chlorophenylaminocarbonyl	**3B**	13	49.55	4.15	7.90	14.93	0.16 ± 0.07	2.05 ± 0.39	52.87	4.42	8.40	14.25
4-Chlorophenylaminocarbonyl	**3C**	0–5	50.32	3.99	8.20	15.14	0.14 ± 0.07	2.50 ± 0.27	52.82	4.11	8.63	15.68
3,5-Dichlorophenylaminocarbonyl	**4C**	0–5	45.16	3.48	6.94	26.14	0.27 ± 0.10	2.18 ± 0.41	47.17	3.63	7.23	25.09
4-Chlorobenzoyl	**5C**	0–5	57.40	4.00	2.37	17.14	0.19 ± 0.02	2.74 ± 0.04	57.21	3.96	2.44	16.94
3,5-Dichlorobenzoyl	**6C**	0–5	49.39	3.15	2.05	28.60	0.23 ± 0.03	2.67 ± 0.06	49.43	3.10	2.11	28.58

[a] Deviations from the calculated values are due to the inorganic content of the sample. This is caused by the high chelating ability of chitosan [10] and is inversely related to the DS (the higher DS, the lower inorganic content).
[b] Calculations based on the ratios C/N and C/H [8].

was allowed to react for 24 h, affording the mixed derivative **1B** (Table 1), with a similar number of alkenoyl groups to the previously described cellulose derivatives. The same procedure was successfully applied in the preparation of the 10-undecenoyl/4-chlorophenylaminocarbonyl derivative **3B**. However, when chitosan **A** was used as the starting material, derivatives **1A** and **3A**, with a lower degree of derivatization, were obtained (Table 1). The lower reactivity of chitosan **A** was attributed to its higher acetyl content.

In order to avoid the variability in the starting material and to obtain an optimized and reproducible method for preparing mixed derivatives, chitosan **A** was subjected to an additional deacetylation process [11], prior to the dissolution–reprecipitation treatment. The acetyl content of the deacetylated product, identified as chitosan **C**, was 0–5%, according to ^{1}H NMR and IR spectra, and elemental analysis (Fig. 2).

Homosubstituted 3,5-dichlorobenzoyl derivatives of the differently acetylated chitosans **A** and **C** were prepared in order to confirm the relation between reactivity and acetyl content (Table 2). This derivative was chosen in order to determine the DS of the resulting polysaccharide more accurately from the Cl/N ratio [8]. Mixed derivatives **1C** and **3C** were also prepared following the same procedure used for **1A**/**B** and **3A**/**B** (Table 1). The results obtained with these derivatives show that the higher the number of acetyl groups in the starting chitosan, the lower its reactivity. However, the variations observed in the amount of acetyl group contained in the starting chitosan do not significantly affect the chromatographic discrimination ability of the chiral selectors prepared from it [6]. Nevertheless, highly substituted derivatives, obtained if a prior deacetylation process is performed, show improved solubility and easier manipulation, facilitating the preparation of chiral supports.

Substitution on the aromatic reagent and its possible influence on reactivity.—Once the derivatizing conditions had been established, derivatives **2C** and **4C**–**6C** were prepared from chitosan **C** (Table 1). All derivatives

Table 2
3,5-Dichlorobenzoylation of chitosans with different acetyl content

Chitosan	% Acetyl groups	Mol of acyl chloride per mol of glucose units	Reaction time	Temperature (°C)	Degree of substitution [a]
A	20	6	8 h	115	0.89 ± 0.05
C	0–5	6	8 h	115	1.95 ± 0.12
A	20	6	16 h	115	1.43 ± 0.07
C	0–5	6	16 h	115	2.24 ± 0.09

[a] Calculations based on elemental analyses. The specified number of remaining acetyl groups is taken into account.

from the **C** series present, as expected, the desired amount of 10-undecenoyl groups and a high DS. Benzoylated chitosans (**5C** and **6C**) show higher DS than the phenylaminocarbonyl derivatives (**1C**–**4C**) because of the difference in the reactivity of aryl isocyanates and benzoyl chlorides. Nevertheless, the substitution of the reagent on the aromatic ring seems to affect the derivatization. Thus, the 4-substituted aryl reagents yielded higher aryl-substituted derivatives than did the corresponding 3,5-disubstituted reagents.

Cass et al. reported [12] preparation of the phenylcarbamate and the 3,5-dimethylphenylcarbamate of chitin. Though neither DS values nor considerations of such an effect are given, calculations from the elemental analyses given by the authors show that a higher DS was obtained for the phenylcarbamate derivative.

This phenomenon, which is not observed for cellulose derivatives, may be attributed to the steric hindrance produced by the aromatic substituents at positions 3 and 5, and to the particular supramolecular structure of chitosan. This hypothesis is consistent with a previous study on the reactivity of branched aliphatic *N*-acylchitosans [9].

3. Experimental

General methods.—^{1}H NMR spectra were measured with a Varian G-300 spectrometer at 70 °C. Samples of non-derivatized chitosan were dissolved in D_2O–CF_3CO_2H using acetone-d_6 as the internal reference (δ 2.10 ppm). Samples of chitosan derivatives (15 mg) were dissolved in 99.6% pyridine-d_5. The downfield peak of the three solvent signals was taken as the internal standard (δ 8.73 ppm). IR spectra were recorded with a Perkin–Elmer FT-IR 1600 spectrometer. Elemental analyses were performed in a CE Instruments apparatus (Mod. EA 1108), using standard conditions, by the Serveis Científico-Tècnics de la Universitat de Barcelona (Spain).

Deacetylation of chitosan.—Commercially available chitosan flakes (5 g) were suspended in 50 mL of 50% NaOH at 100 °C under an argon atmosphere for 2 h, then filtered off and thoroughly washed in distilled water to neutrality. The solid obtained was then treated as described next.

Pretreatment of chitosan.—Commercially available chitosan flakes (2.5 g) or previously deacetylated chitosan were dissolved in 100 mL of 1% HOAc and the solution was filtered. The viscous solution was stirred while 1 M NaOH solution was added until complete neutralization. The precipitate was filtered and exhaustively washed in distilled water. The solid obtained was dispersed in MeOH, filtered and washed in this solvent and then in diethyl ether. Finally, the precipitated chitosan was air-dried at room temperature. The starting chitosans used were characterized at this point by ^{1}H NMR (Fig. 2) and elemental analyses (Table 3).

Preparation of mixed 10-undecenoyl/benzoyl and arylaminocarbonyl derivatives of chitosan.—Previously precipitated chitosan (0.40 g, 2.5 mmol of glucosamine units) was suspended in 30 mL of anhyd pyridine and 15 mmol (6 mmol per mmol of glucosamine units) of the corresponding benzoyl chloride or aryl isocyanate were added. The mixture was stirred at 115 °C until a viscous solution was obtained. 10-Undecenoyl chloride (0.25 g, 1.2 mmol, 0.5 mmol per mmol of glucosamine

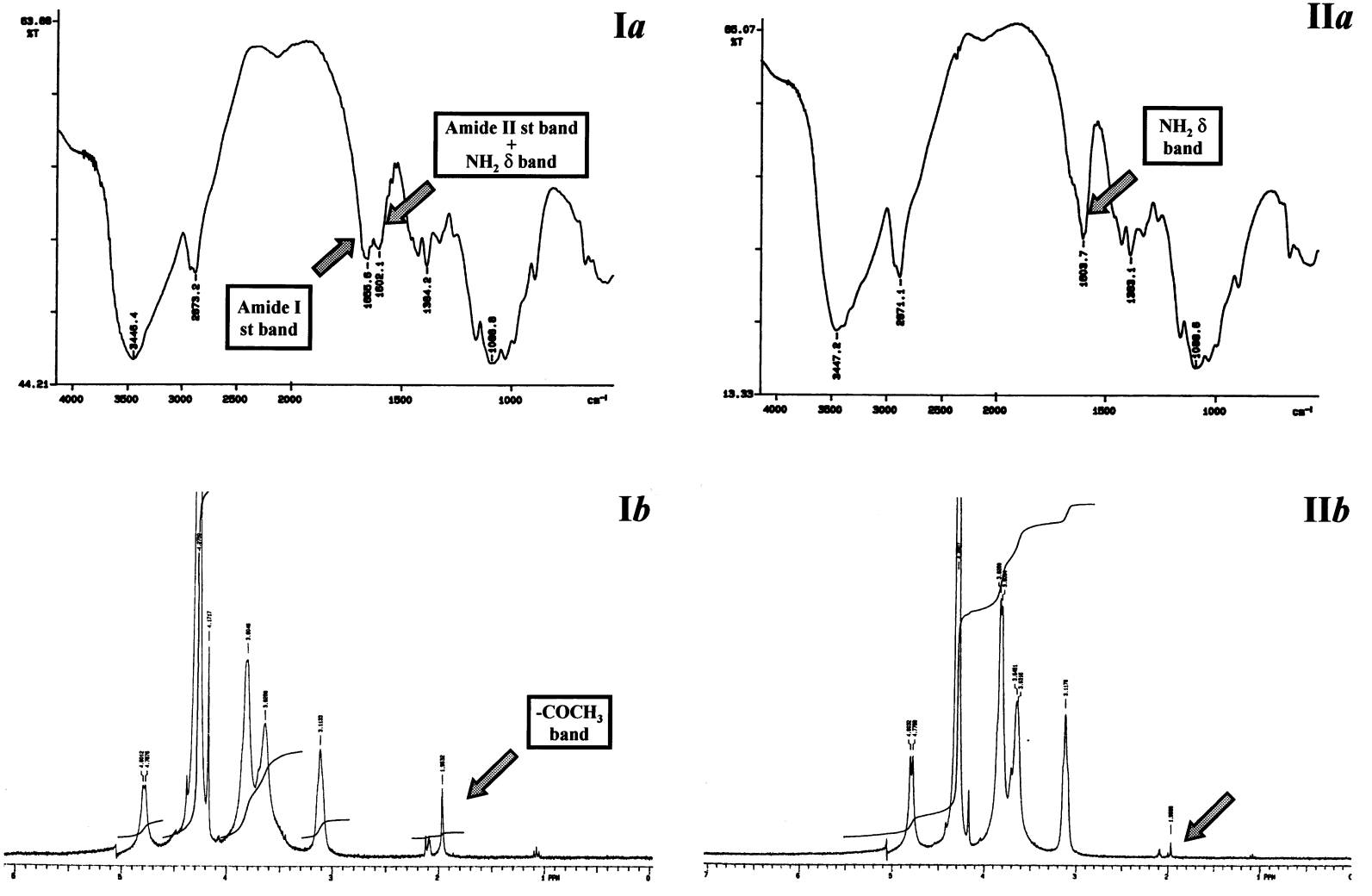

Fig. 2. (I) Spectra of commercial chitosan **A** (*a*: IR; *b*: ^{1}H NMR). (II) Spectra of chitosan **A** after the additional hydrolytic treatment (chitosan **C**) (*a*: IR; *b*: ^{1}H NMR). IR spectra were registered in KBr. ^{1}H NMR spectra were measured in D_2O–CF_3CO_2H, at 70 °C, using acetone-d_6 as internal standard.

Table 3
Elemental analyses of the starting chitosans

Chitosan	Elemental analyses			Calculated acetylation degree [a]
	%C	%H	%N	
A	40.50	6.68	7.39	20%
B	40.73	6.62	7.60	13%
C	39.56	6.85	7.78	0–5%

[a] Calculations based on elemental analyses.

units) was then added, and the solution was allowed to react for 24 h at 115 °C.

The resulting products were isolated as the insoluble fraction in MeOH by filtration, redissolved in $CHCl_3$ or THF, reprecipitated and washed in MeOH. When an arylaminocarbonyl derivative was prepared, the solid obtained was thoroughly washed in hot EtOH in order to remove the *N*,*N*′-bis(aryl)urea formed as a by-product. The derivatives obtained were characterized by ^1H NMR and elemental analysis (Table 1).

4. Conclusions

A reproducible method for the preparation of mixed 10-undecenoyl/phenylaminocarbonyl or benzoyl derivatives of chitosan is described. This method involves a starting deacetylation step in order to avoid the variability in the acetyl content of the starting material. The aromatic derivatizing agent is introduced first. This facilitates the introduction of alkenoyl chains later and the preparation of highly aryl-substituted chiral selectors with an optimum number of 10-undecenoyl groups. The DS of the derivatives obtained also seems to be dependent on the bulkiness of the derivatizing reagents.

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