

Mixed Esters of Chitin

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SYNOPSIS

One of the main obstacles to the wider application of chitin in polymeric materials is its poor tractability due to unfavorable solubility properties. The solubility can be enhanced by introducing bulky acyl residues into the polymer (as in butyrylchitin and valeroylchitin). However, if modification is carried out with shorter-chain carboxylic acids (as in acetylchitin), the solubility remains poor. By substituting the acetyl residues partially by butyryl residues (mixed ester formation), exclusive use of the bulky carboxylic acids can be avoided and yet good solubility is achieved. These relationships were shown by using high molecular weight mixed chitin esters, prepared with methanesulfonic acid as the solvent and catalyst. The mixed chitin esters, varying both in the overall degree of substitution (1.5–1.9) and the molar ratios of butyryl-to-acetyl residues (1 : 0.62 to 1 : 0.72), were characterized by IR spectroscopy, DSC, elemental analysis, and ¹H-NMR spectroscopy (in trifluoromethanesulfonic acid); the latter allowed the degree of substitution to be determined as well as the molar ratio of butyryl-to-acetyl residues. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Chitin is a celluloselike polysaccharide of β -linked 2-acetamido-2-deoxy-D-glucose residues. It is a linear polysaccharide and can be considered as a cellulose derivative, in which hydroxyl groups in the C-2 position are substituted with acetamide groups ($-\text{NH}-\text{CO}-\text{CH}_3$). Chitin is found in fungal and bacterial cell walls, in insect cuticles, and in the shells of crustaceans.¹

The poor solubility of chitin is the major limiting factor in its utilization and in investigating its properties and structure. Despite these limitations, various applications of chitin and modified chitins have been reported in the literature, e.g., as raw material for man-made fibers.²

Attempts have been made to obtain soluble derivatives of chitin^{3–5} by introducing bulky hydrophobic residues as side groups. The substitution of

the hydroxyl groups by ester groups will, in general, increase the solubility by weakening the intermolecular hydrogen bonds. Little is known, however, about using chitin-mixed esters for this purpose, although it can be assumed that due to their more heterogeneous primary structure they should show an enhanced solubility.

This article reports on the preparation and solution properties of some acyl derivatives, especially of the mixed-ester type. The mixed esters were obtained by acylating chitin (I) with various amounts of butyric anhydride and acetic anhydride, delivering acylchitin (II). The chitin derivatives were characterized by IR spectroscopy, DSC, elemental analysis, and ¹H-NMR spectroscopy.

RESULTS AND DISCUSSION

Synthesis

The synthesis of the various acyl derivatives of chitin was performed in methanesulfonic acid (MSA[†]). MSA was found to be a suitable solvent and catalyst

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‡ Abbreviations used in this article: MSA = methanesulfonic acid; TF-MSA = trifluoromethanesulfonic acid; TFA = trifluoroacetic acid; TMS = tetramethylsilane; DS = degree of substitution.

Table I Acylation of Chitin in MSA with Acetic Anhydride/Butyric Anhydride Mixture

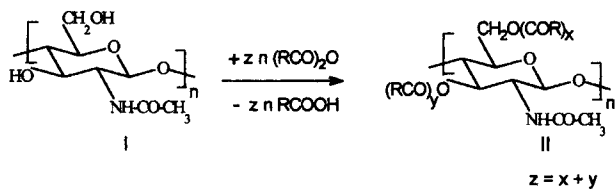
Sample	Chitin ^a (g)	Acetic Anhydride		Butyric Anhydride		Mole Ratio	Yield (g)	η_{inh}^b (dL/g)
		(mL)	(mmol)	(mL)	(mmol)			
Mixed ester-1	3	3.0	31.76	15.0	92.09	1 : 2.90	4.1	0.17
Mixed ester-2	3	4.0	42.35	14.0	85.95	1 : 2.03	4.0	0.18
Mixed ester-3	3	6.5	68.52	11.5	70.60	1 : 1.03	3.8	0.28
Butyryl chitin	3	—	—	18.0	110.15	—	4.1	0.74

^a Three grams of chitin are equivalent to ≈ 30 mmol hydroxyl groups.

^b Measured in anhydrous formic acid at 20°C; $c = 0.5$ g/L.

for the homogeneous acylation reaction even at low temperatures. A mixture of four parts of MSA and six parts of carboxylic acid anhydride for one part of chitin was employed. When smaller amounts of MSA are used, it is difficult to obtain highly acylated derivatives of chitin, owing to the heterogeneity of the reaction mixture. The temperature of the reaction is set to 0–5°C to avoid decomposition reactions of the chitin in MSA. So that acylation can reach completion, the reaction mixture is stored over longer periods (12 h) at low temperatures (–20°C). At this temperature, the micelle structure of the chitin is broken and, accordingly, results in an enhanced reactivity and in a higher degree of substitution (DS).³ These reaction conditions will lead to degrees of substitution in the range of 1.0–1.5.⁵ With a DS estimated at 1.5, the acylchitins were formed in high yields (90–95%). There was, however, slight variation with the molar ratio of acetic anhydride/butyric anhydride (Table I).

The inherent viscosity of pure butyrylchitin prepared in this manner was 2.5 dL/g (acetone, 20°C) and, therefore, much higher than reported in the literature⁶ (0.66 dL/g).



Thermograms

The DSC thermograms of acylchitins prepared in this way show only a large endotherm in the range from 60 to 120°C, due to water evaporation, and do not reveal any transition up to 281°C, in accordance with DSC measurements of chitosan⁷ and other acylchitins.⁸ A water content of 10–15% can be rea-

sonably expected and is commonly found in polysaccharides. Even butyrylchitin does not show any transition at about 258°C, contrary to the results reported in Ref. 8. In polysaccharides, thermal degradation often prevents observation of a glass transition or melting point, owing to the rigidity of the pyranose ring in the polymer backbone.

NMR Spectra

The degrees of substitution (DS) were determined by ¹H-NMR spectroscopy and elemental analysis. The ¹H-NMR spectra of the acylchitins show two well-separated regions with signals. The signals in the range from 0.40 to 2.70 ppm represent the protons belonging to the acetamide group and to the ester groups introduced, whereas the signals in the region from 3.00 to 7.00 ppm belong to the protons of the hexose residues.⁹ An example of such NMR spectra, designated A, is given in Figure 1 and shows the spectrum of mixed ester-2 with assignment of the highfield protons. Assignment of the hexose protons was, however, beyond the scope of this study. The main interest of this work was the determination of the DS and, in the case of the mixed esters, the determination of the butyryl/acetyl ratio in the polymer. For this purpose, only the overall peak area of the signals of the protons attached to the hexose residue is needed as reference (= 7H) for evaluating the peak areas.

As can be seen in Figure 1 (spectrum A), it is not possible to evaluate the DS and the butyryl/acetyl ratio by simple computational integration because of the strong overlapping of the main signals. For this purpose, a computer program¹⁰ was used, which fits given Lorentzian and Gaussian line shapes in an iterative manner onto a measured spectrum. The resulting fitted spectra (see B in Fig. 1) were used for the determination of the DS and the butyryl/acetyl ratio. The results of the NMR

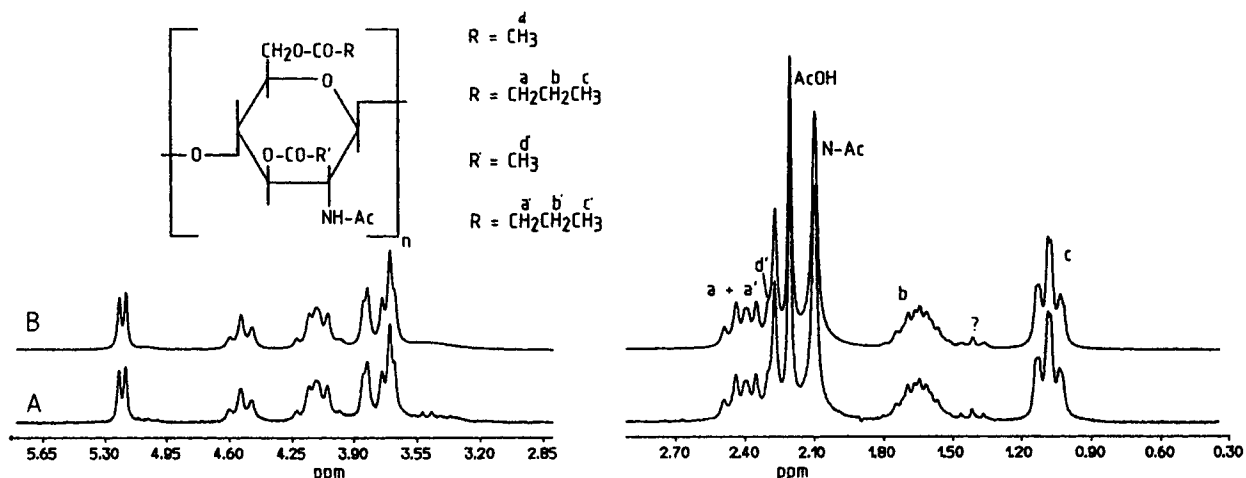


Figure 1 $^1\text{H-NMR}$ spectrum of mixed ester-2: (A) measured; (B) calculated spectrum.

spectroscopic investigation are summarized in Table II and will be discussed below.

A comparison of TF-MSA and TFA as the solvent for NMR spectroscopy shows clearly that TF-MSA is the better solvent for acylchitins. This is indicated by the half-line widths of the signals in the NMR spectra, which are much smaller when the samples are dissolved in TF-MSA. This is a result of the power of TF-MSA in breaking hydrogen bonds. A second reason for the observed effect might be decomposition reactions of the polysaccharide back-

bone. Smaller molecules have a higher mobility in solution, which results in smaller half-line widths in the NMR spectra. Therefore, only TF-MSA was used as the solvent for recording the NMR spectra, although this solvent also has a certain disadvantage when there is water present. The acetamide group and the ester groups will be partly hydrolyzed within 1 day as reported in Ref. 11. This is indicated by the signal at 2.22 ppm (AcOH) referring to free acetic acid. The assignment was confirmed by comparing the spectra of mixed ester-1 (Fig. 2, spectrum

Table II Results of NMR Spectroscopic Compositional Analysis

Signal	Chemical Shift ^a (ppm)	Mixed Ester-1		Mixed Ester-2		Mixed Ester-3		Butyrylchitin	
		Calcd ^b	Measd ^c	Calcd	Measd	Calcd	Measd	Calcd	Measd
Ac—O (3) ^d	2.10	—	0.82	0.48	—	0.41	—	0.81	—
Ac—O (6) ^e	2.28	—	1.46	0.79	—	1.33	—	1.56	—
Ac—O (3 + 6)	—	1.15	2.28	1.27	1.49	1.74	2.21	2.37	—
N—Ac	2.03	3.00	2.37	2.01	3.00	2.48	3.00	1.58	3.00
Ac—OH	2.22	—	1.12	1.16	—	1.69	—	1.71	—
α (3)	2.43	—	1.19	1.09	—	1.00	—	1.11	—
α (6)	2.52	—	0.96	0.83	—	0.88	—	1.06	—
α (3 + 6) ^f	—	2.23	2.15	1.92	2.00	1.88	1.53	2.17	3.00
β	1.38	2.26	2.10	2.33	2.00	1.76	1.53	1.35	3.00
γ	0.56	3.53	3.35	3.39	3.00	2.49	2.30	1.92	4.50
DS (acetyl)	—	—	≥ 0.76	—	—	≥ 0.58	—	≥ 0.79	—
DS (butyryl)	—	—	1.08	—	—	0.94	—	1.09	—
DS (total)	—	—	≥ 1.84	—	—	≥ 1.52	—	≥ 1.88	—

^a Measured in TF-MSA; internal TMS served for shift reference.

^b All calculations in the table are based on the composition of the reaction mixture and on a DS of 1.5 (estimated).

^c First column freshly prepared; second column after 24 h.

^d . . . (3) ester group at position 3.

^e . . . (6) ester group at position 6.

^f Signals marked with α , β , and γ belong to the butyryl residue.

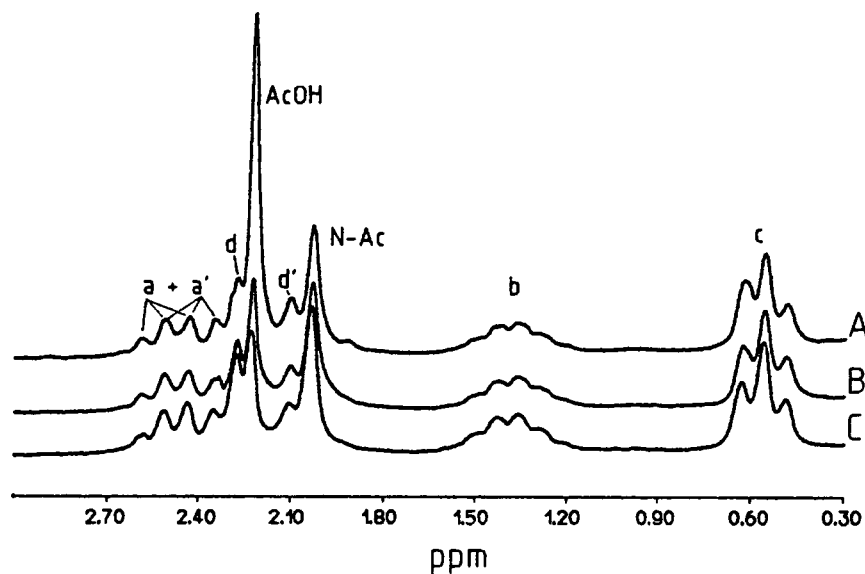


Figure 2 $^1\text{H-NMR}$ spectra of mixed ester-1; (C) freshly prepared sample; (B) same sample after 24 h; (A) same sample with addition of acetic acid.

C) and the spectra of mixed ester-1 with an additional small amount of acetic acid (Fig. 2, spectrum A).

The NMR spectra of the acylchitins show two different signals for acetyl ester groups and butyryl ester groups substituted on positions 3 and 6. For the latter, this effect can be observed only for the α -protons. The chemical shifts are listed in Table II. The signals at higher field are assigned to the

ester groups at position 3, whereas the signals at lower field represent the protons of ester groups at position 6.¹²

The results of the NMR spectroscopic investigation of the chitin-mixed esters lead to the following conclusions:

- (i) The DS of all samples prepared in this study is higher than 1.5, taking into account that

Table III Elemental Analyses and Compositions of Chitin-Mixed Esters

Sample	Acetyl/Butyryl Mol Ratio	Elemental Analyses			Elemental Formula	Mol Weight ^c (g/mol)
		C	H	N		
Mixed ester-1						
Calcd ^a	1 : 2.90	54.05	7.03	4.26	$\text{C}_{14.96}\text{H}_{22.92}\text{N}_1\text{O}_7$	328.79
Calcd ^b		50.08	7.00	4.17	$\text{C}_{14.16}\text{H}_{23.32}\text{N}_1\text{O}_8$	335.58
Found		50.72	6.41	4.21		
Mixed ester-2						
Calcd	1 : 2.03	54.27	6.49	4.31	$\text{C}_{14.66}\text{H}_{22.36}\text{N}_1\text{O}_7$	324.86
Calcd		50.20	6.92	4.27	$\text{C}_{13.7}\text{H}_{22.49}\text{N}_1\text{O}_{7.91}$	327.78
Found		50.45	6.37	4.28		
Mixed ester-3						
Calcd	1 : 1.03	53.35	6.72	4.44	$\text{C}_{14.02}\text{H}_{21.04}\text{N}_1\text{O}_7$	315.60
Calcd		50.69	7.01	4.18	$\text{C}_{14.14}\text{H}_{23.3}\text{N}_1\text{O}_{7.96}$	335.00
Found		49.31	6.14	4.35		

^a Calculations based on a DS = 2 and on a composition corresponding to the reaction mixture.

^b Calculation based on the results of the NMR spectroscopic investigation and a water content of $\sim 7\%$ w/w.

^c Average mol weight per repeating unit.

a part of the free acetic acid results from hydrolysis of the acetyl ester groups. As mentioned before, the acetamide group is much more resistant to hydrolysis than are the acetyl ester groups.

- (ii) The content of acetyl ester groups in the samples is higher than could be expected from the composition of the reaction mixture, which is a result of the higher reactivity of acetic anhydride compared with butyric anhydride. A second reason might be steric hindrance of the butyric residue due to its greater size.
- (iii) Due to differences in reactivity, the acetyl residue is preferably bonded at position 6, whereas the butyric residue is found in the same proportions at positions 3 and 6. This is due to the higher reactivity of hydroxyl groups in position 6 (primary) compared with those in position 3 (secondary) as well

as the higher reactivity of acetic anhydride compared with butyric anhydride.

Elemental Analysis

The elemental analyses agree with the results of NMR spectroscopic studies, especially when the water content ranging from 5 to 10% w/w is taken into account. A water content in this range is commonly found in polysaccharides. Experimentally, this was confirmed by DSC measurements, which show a broad endotherm as a result of water evaporation (see above). The results of elemental analysis are summarized in Table III.

IR Spectra

Infrared spectra of various acylated chitins are depicted in Figure 3. On proceeding with acylation, the absorptions at $1750\text{--}1730\text{ cm}^{-1}$ and $1260\text{--}1210\text{ cm}^{-1}$, which are characteristic for esters of saturated

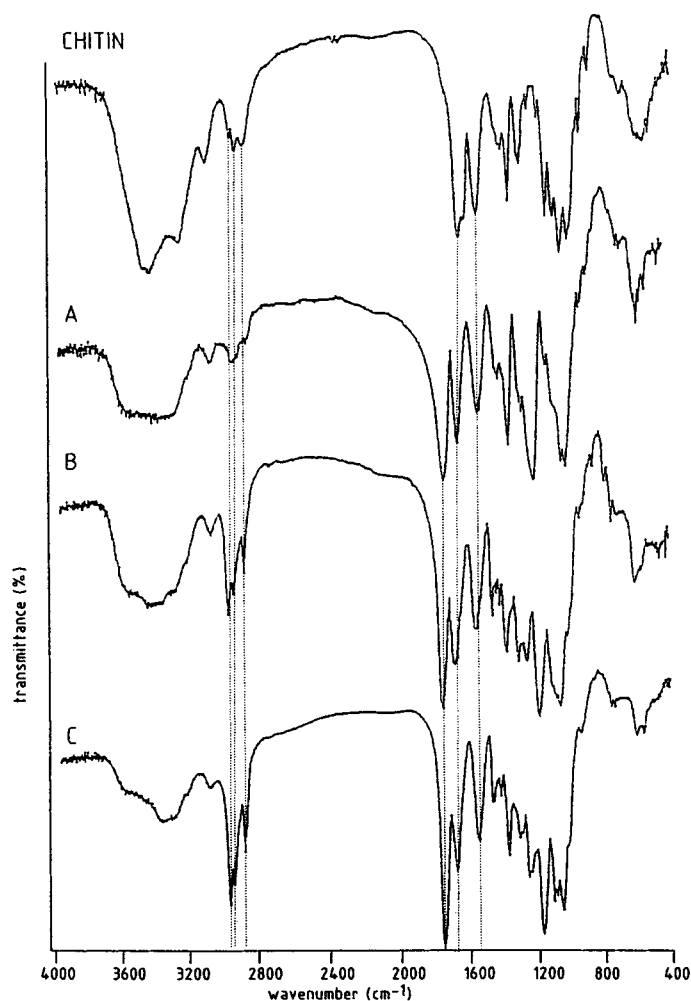


Figure 3 IR spectra of chitin; (A) acetylchitin; (B) butyrylchitin; (C) valeroylchitin¹³.

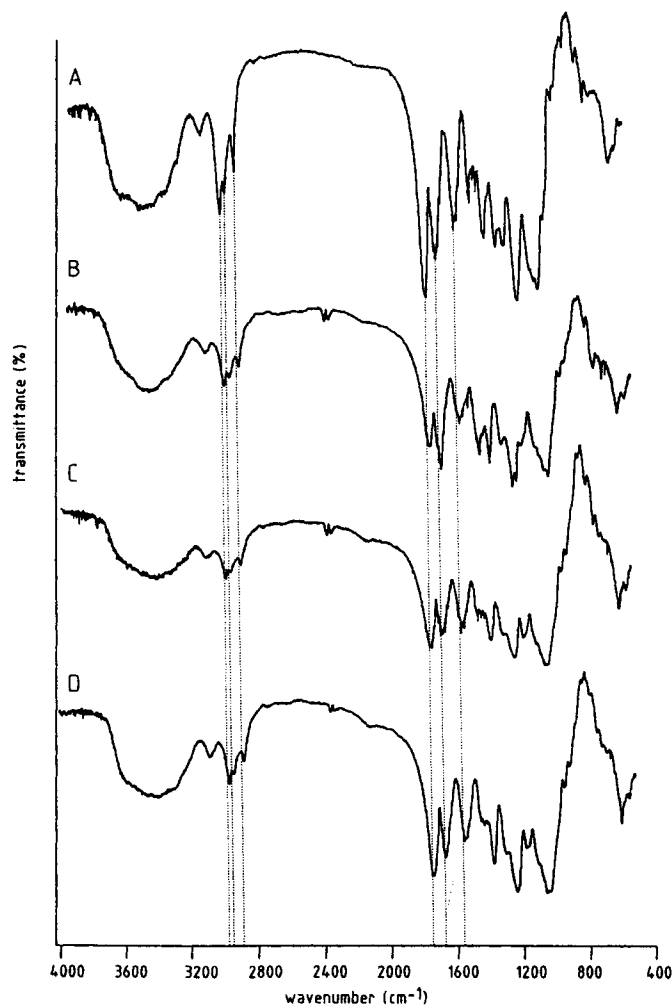


Figure 4 IR spectra of (A) butyrylchitin; (B) mixed ester-1; (C) mixed ester-2; and (D) mixed ester-3.

fatty acids, increased extensively but the absorptions at $3600\text{--}3250\text{ cm}^{-1}$ decreased noticeably. Furthermore, the absorptions due to methylene units also appeared at 2925 and 2850 cm^{-1} . No change was observed in the spectra at $1670\text{--}1650\text{ cm}^{-1}$ (amide I) and at $1560\text{--}1520\text{ cm}^{-1}$ (amide II), showing that the deacetylation process does not occur under the conditions of acylation.

Infrared spectra of the chitin-mixed esters are shown in Figure 4. They are very similar to the spectra of chitins modified by only one carboxylic acid. There is only a difference in the absorptions due to the methylene units in the various chitin esters.

Solubility Properties

Acetylchitin is soluble only in acidic solvents, such as formic acid, dichloroacetic acid, methanesulfonic

acid, or trifluoroacetic acid. The solubility properties of valeroylchitin and butyrylchitin are far better since they show good solubility in common organic solvents. Butyrylchitin is, e.g., soluble in methanol, ethanol, dimethylformamide, dioxane, acetone, and tetrahydrofuran and in acidic solvents such as formic acid or acetic acid. A higher DS gives rise to better solubility. Acylchitins with DS lower than 1.5 are, e.g., only present in a swollen state in acetone.

Although butyrylchitin and acetylchitin are both soluble in formic acid, the mixed ester acetyl-butyryl-chitin is not only soluble in formic acid but also in mixtures of formic acid with polar solvents such as dimethylformamide, methanol, or acetone. A solution of mixed ester-2, e.g., in formic acid can be blended with 10 times its volume of dimethylformamide and five times its volume of methanol or acetone, without precipitation of the polymer.

EXPERIMENTAL

Materials

Powdered chitin, isolated from shrimp shells by a modified standard extraction procedure,² was used for all experiments. Methanesulfonic acid (MSA) and the anhydrides of acetic acid and butyric acid were purchased from Merck & Co., Darmstadt, Germany.

Acylation of Chitin

One part of powdered chitin was added to a mixture of four parts MSA and six parts of carboxylic acid anhydrides. In the case of mixed esters, the products were obtained by varying the amount of butyric anhydride and acetic anhydride. The reaction mixture was stirred for 2–3 h at 0–5°C and stored at –20°C overnight to complete the reaction. The products were precipitated in large quantities of cracked ice, filtered off, and washed with distilled water. The samples were subsequently suspended in distilled water, neutralized with ammonia water, and then boiled for a few minutes to neutralize any traces of acids. The acylchitins or mixed esters were collected by filtration, washed with distilled water, and dried *in vacuo* (yield > 90%).

Measurements

The IR spectra were recorded by KBr disc method on a Nicolet 20 SXB FTIR spectrometer. The ¹H-NMR spectra were obtained on a Bruker AC 100 at a field strength of 2.35 T, using 5 mm o.d. sample tubes. Samples of about 4 mg were dissolved in 1 mL of deuterated trifluoroacetic acid (TFA) or 1 mL of deuterated trifluoromethane sulfonic acid (TF-MSA). Tetramethylsilane (TMS) was used as an internal standard for shift referencing. The inherent and intrinsic viscosities were measured at 20°C in anhydrous formic acid and acetone, respectively, with an automated Ubbelohde viscosimeter. The DSC thermograms were registered with a Per-

kin-Elmer DSC 4 (heating rate 20°C/min, N₂ atmosphere).

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