



# One-pot synthesis of thermoplastic mixed paramylon esters using trifluoroacetic anhydride



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## ABSTRACT

Mixed paramylon esters prepared from paramylon (a storage polysaccharide of *Euglena*), acetic acid, and a long-chain fatty acid by one-pot synthesis using trifluoroacetic anhydride as a promoter and solvent were shown to have thermoplasticity. Size exclusion chromatography indicated that the mixed paramylon esters had a weight average molecular weight of approximately  $4.9 - 6.7 \times 10^5$ . Thermal analysis showed that these esters were stable in terms of the glass transition temperature ( $>90^\circ\text{C}$ ) and 5% weight loss temperature ( $>320^\circ\text{C}$ ). The degree of substitution of the long alkyl chain group, a dominant factor determining thermoplasticity, was controlled by tuning the feed molar ratio of acetic acid and long-chain fatty acid to paramylon. These results implied that the one-pot synthesis is useful for preparing structurally-well defined thermoplastic mixed paramylon esters with high molecular weight.

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## 1. Introduction

The creation of products from microalgae has recently attracted particular attention, mainly because of their ability to fix carbon dioxide and metabolize carbon sources in wastewater (Chisti, 2007). The target products that have been mostly studied are bio-fuels with the expectation of reducing dependency on fossil fuel (Banerjee, Sharma, Chisti, & Banerjee, 2002; Kaya et al., 2011; Mata, Martins, & Caetano, 2010; Tucci, Vacula, Krajcovic, Proksch, & Martin, 2010; Yamane et al., 2013). Despite intensive efforts to make the production more efficient, further developments for reducing production cost are required for practical use.

Our efforts in this area have focused on the creation of high-value-added organic materials from polysaccharide of *Euglena* (Shibakami, Sohma, & Hayashi, 2012; Shibakami, Tsubouchi, Nakamura, & Hayashi, 2013). This polymer, which is referred to as paramylon, is a storage polysaccharide in which glucose units are linked by  $\beta$ -1,3-bonds (Barras & Stone, 1968). Since paramylon is produced in significant quantities by using readily cultured

*euglenoid* alga (Barsanti, Vismara, Passarelli, & Gualtieri, 2001; Santek, Friehs, Lotz, & Flaschel, 2012), this polymer is a promising biomass. We recently reported the creation of *Euglena*-based thermoplastics from mixed paramylon esters (Shibakami, Tsubouchi, & Hayashi, 2014). The procedure we used for synthesizing these esters is two-pot esterification in which a long alkyl chain and acetyl groups are sequentially introduced into the glucose unit of paramylon. Although this two-pot synthetic procedure is not complicated, the development of a one-pot synthetic method may lead to *Euglena*-based thermoplastics becoming more practical.

Our ultimate goal is to establish a one-pot method for synthesizing thermoplastic mixed paramylon esters as a component of mechanically and thermally stable *Euglena*-based plastics. The immediate objective of the work reported here was to find a one-pot synthetic method that produces paramylon with thermoplasticity. In particular, we examined the feasibility of one-pot esterification using trifluoroacetic anhydride (TFAA). While the preparation of several polysaccharide esters using this one-pot synthesis has been reported (Marubayashi, Yukinaka, Enomoto-Rogers, Takemura, & Iwata, 2014; Yang & Montgomery, 2008; Yang, Ding, & Montgomery, 2009), there has been no study of paramylon esters. In this paper, we describe the suitability of one-pot esterification for producing thermoplastic mixed paramylon esters

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comparable to those prepared by two-pot esterification. We also briefly compare the structural and thermal properties of mixed paramylon esters prepared using one-pot and two-pot synthesis.

## 2. Experimental

### 2.1. General methods

$^1\text{H}$  nuclear magnetic resonance (NMR) spectra were recorded on Bruker AVANCE 500 and JEOL JNM-ECX400 spectrometers. Quantitative  $^{13}\text{C}$ NMR spectrum was obtained using the inverse-gated decoupling method (65,536 cumulative times). Fourier transform infrared (FT-IR) spectra were recorded using a JASCO FT/IR-480ST spectrophotometer equipped with an attenuated total reflectance accessory (ATR Pro 400-S, ZnSe prism, JASCO) with a resolution of  $4\text{ cm}^{-1}$ . The melting behavior of the products was observed using a Yanako MP-500D melting-point apparatus. Optical microscopic observation was performed with a Leica DMRE microscope.

All chemicals and reagents were used without further purification. Paramylon particles were obtained from *Euglena gracilis* in accordance with a previously reported method (Shibakami et al., 2012). The degree of substitution (DS) values, i.e., the average number of functional groups attached to a glucose unit, were determined by comparing the integral values of the methyl protons of the long alkyl chain and acetyl group with those of the glucosidic protons in the  $^1\text{H}$ NMR spectrum. The calculation of DS values takes account of the non-functionalized OH groups that have signals appearing in the glucosidic proton range. The equations used for the calculation were as follows.

$$3\text{DS}_{\text{ace}} \times \frac{7.0}{7.0 + \text{DS}_{\text{H}}} = a$$

$$3\text{DS}_{\text{lac}} \times \frac{7.0}{7.0 + \text{DS}_{\text{H}}} = b$$

$$\text{DS}_{\text{ace}} + \text{DS}_{\text{lac}} + \text{DS}_{\text{H}} = 3.0$$

where  $\text{DS}_{\text{ace}}$ ,  $\text{DS}_{\text{lac}}$ , and  $\text{DS}_{\text{H}}$  represent the DS of acetyl, long alkyl chain (octanoyl, decanoyl, lauroyl, myristoyl, palmitoyl, or stearoyl), and proton, respectively;  $a$  and  $b$  are the relative integration value due to the methyl group of acetyl and long-chain alkyl group, respectively, when the total glucosidic protons were set to 7.0.

### 2.2. Synthesis of mixed paramylon esters

#### 2.2.1. Paramylon acetate myristate prepared using one-pot synthesis in DMAc/LiCl from paramylon, acetic anhydride, and myristoyl chloride (1)

To a homogeneous solution of paramylon (10.00 g, 61.68 mmol) and lithium chloride (LiCl) (7.84 g, 185.00 mmol) in N,N-dimethylacetamide (DMAc) (500 mL) prepared by heating at  $\sim 120^\circ\text{C}$  for 0.5 h were added dropwise at room temperature triethylamine ( $\text{NEt}_3$ ) (43.3 mL, 308.51 mmol) and a solution made from DMAc (500 mL) and myristoyl chloride (8.4 mL, 30.84 mmol). This mixture was heated at  $120^\circ\text{C}$  under a nitrogen atmosphere for 3 h and then cooled to  $70^\circ\text{C}$ . Next,  $\text{NEt}_3$  (300 mL, 2.15 mmol), acetic anhydride (240 mL, 2.54 mmol), and DMAc (500 mL) were added to the solution. After mechanical stirring at  $70^\circ\text{C}$  for 5 h and at room temperature for 14 h, a mixture of methanol (1500 mL) and water (140 mL) was added to precipitate a solid. After removal of supernatant by filtration, the solid was washed with water (1600 mL) by mechanical stirring for 1.5 h. Air-drying for 4 h and subsequent vacuum-drying at  $90^\circ\text{C}$  for 1 h produced a dark orange-colored solid (20.59 g). A part of this solid was purified as follows. To a solution made from the solid (1.03 g) and

chloroform (25 mL) was added methanol (250 mL) to precipitate a yellowish solid. This procedure was repeated two times. Air-drying for 16 h and subsequent vacuum-drying at  $90^\circ\text{C}$  for 2 h produced paramylon acetate myristate (**1**) as a pale yellowish solid (734 mg, 2.62 mmol, yield 84.3%).  $^1\text{H}$ NMR ( $\text{CDCl}_3$ )  $\delta$  5.04–4.71(m), 4.53–4.13(m), 4.11–3.96(m), 3.87–3.33(m), 2.47–2.33(m), 2.12(s), 2.06(s), 2.00(s), 1.60(m), 1.26(s), 0.88 (t,  $J \equiv 6.8$ ). FT-IR ( $\text{cm}^{-1}$ ) 2925, 2852, 1742, 1636, 1434, 1368, 1213, 1190, 1119, 1037, 889.

#### 2.2.2. Paramylon acetate myristate prepared using one-pot synthesis and TFAA from paramylon (1 eq), acetic acid (10 eq), and myristic acid (6 eq) (2)

To a round-bottomed flask containing TFAA (8.0 mL) were added acetic acid (710  $\mu\text{L}$ , 12.41 mmol) and myristic acid (1.499 g, 7.48 mmol) at  $50^\circ\text{C}$ . After stirring for 5 min, paramylon (200 mg, 1.23 mmol) was added to the solution under a nitrogen atmosphere and stirred for 1 h. The resulting homogeneous solution was poured into a saturated sodium hydrogen carbonate aqueous solution (400 mL). A white solid was obtained from this solution by filtration. The solid was washed with water (80 mL) for 15 min and methanol (80 mL) for 15 min by mechanical stirring (two times), followed by vacuum-drying at  $90^\circ\text{C}$  for 2 h to yield paramylon acetate myristate (**2**) (523 mg, 1.15 mmol, yield 93.5%).  $^1\text{H}$ NMR ( $\text{CDCl}_3$ )  $\delta$  4.95–4.65(m), 4.43–4.14(m), 4.07–3.91(m), 3.81–3.52(m), 2.41–2.21(m), 2.12(s), 2.06(s), 2.00(s), 1.59(m), 1.26(s), 0.88 (t,  $J \equiv 6.6$ ). FT-IR ( $\text{cm}^{-1}$ ) 2918, 2851, 1745, 1456, 1368, 1213, 1146, 1111, 1038, 1026, 890, 799, 721, 631.

#### 2.2.3. Paramylon acetate myristates prepared using one-pot synthesis and TFAA from paramylon, acetic acid, and myristic acid with different feed molar ratio (3–5)

Procedures similar to those described for the preparation of **2** were used to obtain paramylon acetate myristate (**3–5**) using different feed molar ratio (i.e., 1:11:5, 1:12:4, and 1:14:2 (paramylon (glucose unit): acetic acid: long-chain fatty acid)). Yields (%) were 78.1 (**3**), 71.4 (**4**), and 72.7 (**5**).

#### 2.2.4. Mixed paramylon esters prepared using one-pot synthesis and TFAA from paramylon (1 eq), acetic acid (12 eq), and myristic acid (4 eq) (6–10)

Procedures similar to those described for the preparation of **2** were used to obtain mixed paramylon esters (**6–10**) made from paramylon, acetic acid, and different long-chain fatty acid (octanoic acid, decanoic acid, lauric acid, palmitic acid, and stearic acid) with a feed molar ratio of 1:12:4. Yields (%) were 92.5 (**6**), 88.4 (**7**), 88.3 (**8**), 91.1 (**9**), and 92.0 (**10**).

#### 2.2.5. Paramylon acetate myristate prepared using two-pot synthesis in DMAc/LiCl from paramylon, acetic anhydride, and myristoyl chloride (11)

Two-pot synthesis of paramylon acetate myristate was done using a method previously reported (Shibakami et al., 2014). To a homogeneous solution of paramylon (10.00 g, 61.68 mmol) and LiCl (7.84 g, 185.00 mmol) in DMAc (500 mL) prepared by heating at  $\sim 120^\circ\text{C}$  for 0.5 h were added dropwise at room temperature  $\text{NEt}_3$  (43 mL, 308.38 mmol) and a solution made from DMAc (500 mL) and myristoyl chloride (8.4 mL, 30.9 mmol). This mixture was heated at  $120^\circ\text{C}$  under a nitrogen atmosphere for 3 h. Methanol (1200 mL) was then added to precipitate a solid. After removal of supernatant by centrifugation, the solid was washed with a mixture of methanol (600 mL) and chloroform (300 mL) for 1 h. The wet solid separated by centrifugation was air-dried for 15 h and subsequently vacuum-dried at  $90^\circ\text{C}$  for 2 h to produce paramylon myristate as a solid (15.00 g).

To a homogeneous solution containing DMAc (1500 mL), LiCl (6.50 g, 153.46 mmol), and paramylon myristate (15.00 g), which

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