Chemoenzymatic Synthesis of Sucrose-Containing Aromatic Polymers

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Abstract: A chemoenzymatic approach was developed to prepare sucrose-containing aromatic polymers. The protease from *Bacillus licheniformis* catalyzed the transesterification of sucrose with a diester of terephthalic acid in pyridine to give the mono- and diester products. At 45°C, >70% of sucrose was consumed after 1 day and sucrose diester began to form after 6 days when >95% of sucrose had been converted to sucrose monoester. The final yield of sucrose diester after 20 days was 13.8%. The sucrose monoester was identified as sucrose 1′-terephthalate and the diester products consisted of sucrose 6,1′-diterephthalate and sucrose 6′,1′-diterephthalate in a ratio of 2:1. The sucrose diester products were polymerized with ethylene-glycol and ethylene-diamine to give poly(ethylene-terephthalate) and poly(ethylene-terephthalamide), with sucrose contained in the polymer backbone. The polycondensation reactions were carried out in dimethylsulfoxide (DMSO) at 70°C using zinc acetate as a catalyst. The sucrose-containing polyester and polyamide were obtained at 65% yield for 24 h and at 73% yield for 12 h, respectively. End-group analysis of the polymers by ^{13}C-NMR or ^{1}H-NMR in DMSO provided a number average molecular weight of 3200 and 4300 Da, respectively. Structural analyses of the polymers were performed with ^{1}H-NMR, ^{13}C-NMR, and FTIR. On the basis of ^{13}C-NMR, acylation of the C1′, C6, and C6′ hydroxyls were maintained in the polymer backbones. *Biotechnol Bioeng* **72**: 541–547, 2001.
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INTRODUCTION

The potential of carbohydrates as feedstocks for chemicals has been demonstrated but not yet significantly commercially realized, except in a few instances, because regioselective modification (e.g., acylation of sugars) is a fundamental and difficult task in organic chemistry. For example, production of oligoesters (e.g., di-, tri-, and tetra-) of sugars is a difficult problem in organic chemistry due to the abundance of hydroxyl groups in sugar molecules and the similar reactivity of most of them. Even preferential acylation of primary over secondary hydroxyl groups can only rarely be efficiently carried out with free sugars; this process usually requires protected sugars, thereby necessitating cumbersome protection and deprotection steps (Haines, 1976, 1981).

Enzymatic regioselective acylation of sugars offers an alternative to the poor selectivity of chemical synthesis. In organic media, enzymes retain their inherently keen specificity and, in recent years, great interest has been focused on the regioselective acylation of sugar-based polyalcohols using hydrolytic enzymes. The regioselectivity of the enzyme can be used for the construction of sugar-based polymers having highly regular structures, and incorporation of sugars into traditional polymers (e.g., polyesters, -amides, -ols, -acrylates, etc.) may significantly extend the application of such materials into areas such as catalysts and reagents for organic synthesis; adsorbents with specific chirality, hydrophilicity, and water absorbency; and biodegradabilities. For example, the selectivity of enzymes enables sucrose to react as if it were a diol, even though it contains eight free hydroxyl groups. Patil et al. (1991a) used alkaline protease from a *Bacillus* sp. to incorporate sugars into polyester backbones. They utilized the protease to catalyze the polycondensation of sucrose with di(2,2,2-trifluoroethyl)-adipate in anhydrous pyridine. Only the 6 and 1′ positions of sucrose were acylated in the resulting polymer and water-soluble polymers with $M_w = 2100$ and $M_n = 1600$ were prepared.

Complete enzymatic synthesis of polymers is compelling, yet the rate of enzymatic catalysis is universally slow and the molecular weights of the polymers are not yet as high as is desirable. A far more efficient approach would be to use enzymes only for the highly selective step(s) in polymer synthesis (such as monomer preparation) and to employ conventional chemical catalysts for the bulk polymer synthesis (Blinkovsky and Dordick, 1993; Dordick, 1992). Such a chemoenzymatic approach is ideally suited for the synthesis of sugar-based polymers as the most successful group of polymers prepared by chemoenzymatic means are

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poly(acrylate)s that contain pendant functional groups (Dordick et al., 1994; Martin et al., 1992). In this case, enzymatic synthesis of acrylic acid compounds is performed by transesterification of sugars with an acrylate ester using hydrolases in organic media and the monomers are subsequently subjected to free-radical bulk polymerization using conventional catalysts. Sugar-based polymers can be also prepared by non–free-radical means, and it was reported by Patil et al. (1991b) that acylation of sucrose with an excess of bis(2,2,2-trifluoroethyl)-adipate catalyzed in pyridine by the protease from *Bacillus* sp. resulted in the synthesis of sucrose 6,1′-bis(trifluoroethyladipate). Also, polymerization of this product with ethylene-diamine gave a water-insoluble, amorphous material with $M_w = 8100$ and $M_n = 4800$.

In our previous studies (Park et al., 1994, 1995), we described the first successful enzymatic synthesis of aromatic polyesters in organic solvents. Oligomers with average molecular weights ranging from 400 to 1400 Da were obtained using aliphatic diols and aromatic diesters or aliphatic diesters and aromatic diols. Our previous success in developing enzymatic synthesis of aromatic polyesters was extended to the synthesis of sugar-containing aromatic polymers by a chemoenzymatic method. The key step in the chemoenzymatic method for the synthesis of sugar-containing aromatic polymers is the highly selective enzymatic transesterification of a sugar with aromatic diesters. This is difficult to perform chemically, due to lack of control in the acylation step, and would result in multiple modified sugars. However, it was found that Optimase M-440, a protease from *Bacillus licheniformis* can also efficiently catalyze the transesterification of aromatic diesters and sugar alcohols. In this work, we describe the chemoenzymatic synthesis of two aromatic polymers containing sucrose. The first is a polyester and the second is a polyamide.

MATERIALS AND METHODS

Materials

Protease from *Bacillus licheniformis* (Optimase M-440) was obtained from Solvay Enzyme Co. (Elkhart, IN). Potassium bromide, sucrose, and Sigma Sil-A were obtained from Sigma Chemical Co. (St. Louis, MO), and Sigma Sil-A was used for the preparation of TMS derivatives of sugars. Ethylene-glycol, ethylene-diamine, and zinc acetate were obtained from Aldrich Chemical Co. (Milwaukee, WI). Bis(2,2,2-trifluoroethyl)-terephthalate was synthesized from terephthaloylchloride and 2,2,2-trifluoroethanol by following the general methodology and had the same characteristics as previously described (Park et al., 1994). All other chemicals were obtained from commercial suppliers and were of analytical grade.

Analytical Methods

All sugar derivatives in this work were determined by the same gas chromatography method as previously described (Park et al., 1994) using a 10-m Alltech AT-1 capillary column packed with polydimethylsiloxane (helium as carrier gas at 15 mL/min, detector and injector port temperatures 300°C), and the temperature of the column was increased from 250° to 300°C at 5°C/min. All reaction mixtures were subjected to precolumn derivatization with Sigma Sil-A.

In addition to gas chromatography, all products were also analyzed by TLC using precoated silica gel 60 F_{254} glass sheets (Merck) and ethyl-acetate:methanol:water (18:1.25:1) as a developing solvent. The spots were visualized with a UV lamp at 254 nm and were also developed by spraying with 10% H_2SO_4 in ethanol followed by heating.

Fourier-transform infrared (FTIR) spectra were obtained at 4-cm^{-1} resolution on a Bomem spectrometer. The spectrometer was equipped with 1-mm DTGS detector. The solid samples used for the FTIR studies were compression-molded with KBr powders and the liquid sample was spread over the KRS5 window. At least 20 scans were signal-averaged and stored on a magnetic disk system for further analysis.

The positions of acylation in all enzymatically prepared compounds were established by ^{13}C-NMR (Brüker AMX 500), and ^{1}H-NMR and ^{13}C-NMR were carried out for structural and end-group analyses for determination of molecular weight.

Enzymatic Reaction

The initial concentrations of bis(2,2,2-trifluoroethyl)-terephthalate (TFE-terephthalate) and sucrose were 0.1 and 0.4 *M*, respectively. TFE-terephthalate should be used in excess to improve the yield of diester (relative to monoester). Both pyridine and the enzyme were dried prior to use to eliminate hydrolysis of the sucrose diester.

Small-Scale Reactions

The reactions were initiated by the addition of 100 mg/mL Optimase M-440 to 2 mL of pyridine containing the substrates in a 10-mL screw-cap vial and the suspension was placed in a shaking incubator at 250 rpm. Periodically, 50-μL aliquots were withdrawn and analyzed by gas chromatography. All samples were derivatized with Sigma Sil-A before injection. The reaction was terminated by filtering out the enzyme.

Large-Scale Reactions

For gram-scale synthesis of sucrose esters, 100 mg/mL of Optimase M-440 was added to pyridine containing the substrates to generate a 100-mL reaction mixture in a 0.5-L round-bottomed flask. The progress of the reaction was monitored by TLC and GC. The reaction was continued for 20 days, the enzyme was removed by centrifugation, and the solvent was evaporated under vacuum. The sucrose esters were then isolated by silica gel flash chromatography using

an eluent consisting of ethyl-acetate:methanol:water (18: 1.25:1).

Polymerization of Sucrose Diester

Sucrose-containing aromatic polymers were prepared via solution polycondensation at 70°C in a glass reactor provided with a nitrogen inlet and a magnetic stirrer. The resulting polymers were recovered by freeze drying dimethylsulfoxide (DMSO) and then washed with acetone to remove zinc acetate and unreacted monomers.

Polyester

For the synthesis of sucrose-containing poly(ethylene-terephthalate), polymerization was carried out by adding 14.9 mg (0.12 *M*) of ethylene-glycol to 0.19 g (0.12 *M*) of sucrose diterephthalate in 2 mL of DMSO. Zinc acetate (0.2%) was added and the solution was stirred at 70°C for 24 h.

Polyamide

Sucrose-containing poly(ethylene-terephthalamide) synthesis was carried out by dissolving 14.4 mg (0.12 *M*) of ethylene-diamine and 0.19 g (0.12 *M*) of sucrose diterephthalate in 2 mL of DMSO. Zinc acetate (0.2%) was added and the solution was stirred at 70°C for 12 h.

RESULTS AND DISCUSSION

Enzymatic Synthesis of Sucrose Ester

In the presence of Optimase M-440, the transesterification reaction between sucrose and TFE-terephthalate took place readily and there was no appreciable conversion detected without enzyme. When the reaction was performed with the inactivated enzyme that had been treated in pyridine at 100°C, no reaction was detected. This indicates that the reaction was caused by the enzyme activity. The various forms of sucrose moiety in the enzymatic transesterification were monitored by gas chromatography (GC) at two representative temperatures (30° and 45°C) (Fig. 1). Each plot shows the fraction of the starting sucrose that was unchanged, that was transesterified once, or that was transesterified twice as the reaction progressed.

At 30°C, the fraction of sucrose drops rapidly at the beginning of the reaction. More than 70% was consumed after 1 day and about 98% was utilized by 10 days. The number of sucrose monoester moieties rose rapidly at the beginning of the reaction when the concentration of sucrose was high, but its slope decreased markedly after 2 days when about 85% of the sucrose had reacted. It reached a maximum at 10 days after the start of the reaction and then began to decrease very slowly. The sucrose diester was obtained in 7% yield after 20 days.

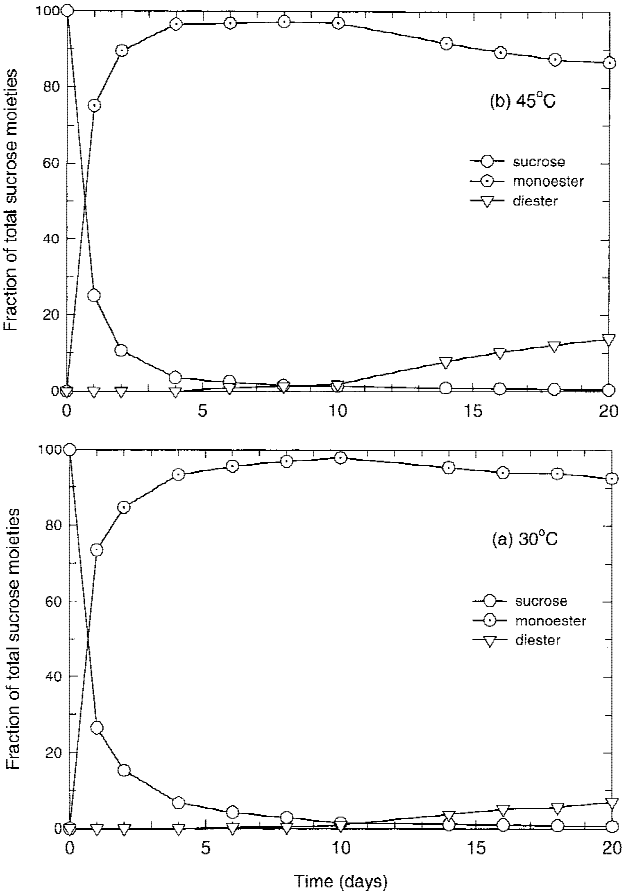


Figure 1. Time profiles of sucrose and sucrose moieties.

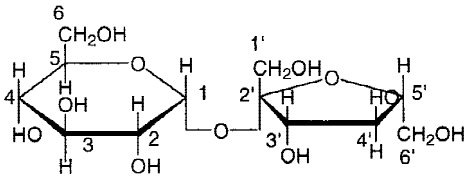
At 45°C, the curve is quite similar, but the formation of sucrose diester was faster and the final yield of sucrose diester after 20 days was about twofold greater (13.8%). In both cases, sucrose diester began to form after 6 days when >95% of sucrose had been converted to sucrose monoester. The results in Figure 1 indicate that a higher temperature (45°C) was much better for the formation of diester, whereas the formation of monoester depended little on the reaction temperature.

To do a preparative synthesis of the sucrose esters, large-scale reactions were carried out at 45°C, as described earlier. After 20 days, the reaction was terminated with filtering of the enzyme and evaporation of the organic solvent. The residual solids were loaded onto a silica column, and sucrose esters were separated into monoester and diester. No triester was ever formed, as evidenced by TLC and GC.

To determine the site of sucrose acylation, ^{13}C-NMR analysis of the sucrose ester products was carried out according to the general strategy developed by Yoshimoto et al. (1980). The nuclear magnetic resonance (NMR) spectra of the sucrose esters were compared with those of an unmodified sucrose (Table I). As detailed in Table I, the monoester product shows a distinct downfield shift in the C1′ carbon with a concomitant upfield shift in C2′ carbon. When the monoester product was analyzed by gas chromatography, small amounts (<5%) of different monoester iso-

Table I. Chemical shifts (ppm) of sucrose monoterephthalate and sucrose diterephthalate in DMSO.

Carbon number	Sucrose	Sucrose monoterephthalate	Sucrose diterephthalate
2′	104.0	102.2	102.2
1	91.7	92.3	92.1
5′	82.6	82.9	83.0 (67%)
			79.4 (33%)
3′	77.1	77.2	77.1
4′	74.3	73.7	73.7
3	72.9	73.1	73.1
5	72.8	73.0	72.6 (33%)
			70.2 (67%)
2	71.6	71.5	71.3
4	69.9	70.0	69.8
1′	62.0	63.9	63.7
6′	62.1	62.3	65.2 (33%)
			62.1 (67%)
6	60.5	60.6	62.4 (67%)
			60.1 (33%)



mers were also indicated by minor peaks present in the spectra. ^{13}C-NMR data indicate that the enzymatic acylation of sucrose with TFE-terephthalate gave almost exclusively sucrose 1′-terephthalate for the monoester, and it was reported by Rich et al. (1995) that 1′-acylation is favored in solvents that can solvate sugars (such as pyridine) as the glucose moiety is exposed to the medium, whereas 6-acylation leaves the entire sucrose molecule buried within the enzyme's binding pocket. Thus, 1′-acylation is sterically more favorable than 6-acylation. This preferential acylation of sucrose at the 1′-OH is distinct from chemical acylation. Chemical acylation with acid chlorides, acid anhydrides, acyl azides, acyl cyanides, and *N*-acylimidazoles results in an acylation preference of 6-OH ≥ 6′-OH > 1′-OH > secondary-OH (Clode et al., 1985; Haines, 1976; Ogawa and Matsui, 1977).

The diester product showed clear downfield shifts in carbons C1′, C6, and C6′ with upfield shifts in carbon C2′, C5, and C5′. These chemical shifts have enabled us to assign structures to the diester products with the diester products identified as a mixture of sucrose 6,1′-diterephthalate and sucrose 6′,1′-diterephthalate in a ratio of 2:1. In our earlier work (Park et al., 1996), it was noted that the reaction product was sucrose 6,1′-diterephthalate alone. However, this detailed study has revealed the presence of two diester products: sucrose 6,1′-diterephthalate and sucrose 6′,1′-diterephthalate. Hence, sucrose was acylated at the 1′ position followed by acylation at the 6 position or 6′ position (Fig. 2). It is noteworthy that 33% of the diester product was sucrose 6′,1′-diterephthalate. It is well known that the 6′-OH of sucrose is involved in a strong intramolecular hydrogen bond with the 5-oxygen of the glucose ring, thereby making the 6′ position not readily available for enzymatic acylation (Chauvin et al., 1993; Khan, 1976). In fact, no report of enzymatic 6′-OH acylation is available in the literature. Acylation at the 6′ position, which has been considered much less favorable than even the secondary hydroxyl group, was also observed in our another study (Park et al., 2000), and it can be pointed out that Optimase M-440 is very distinctive from other enzymes with regard to its catalytic power to acylate sucrose at the 6′-OH position as well as its efficiency of use for 6-OH acylation of sucrose.

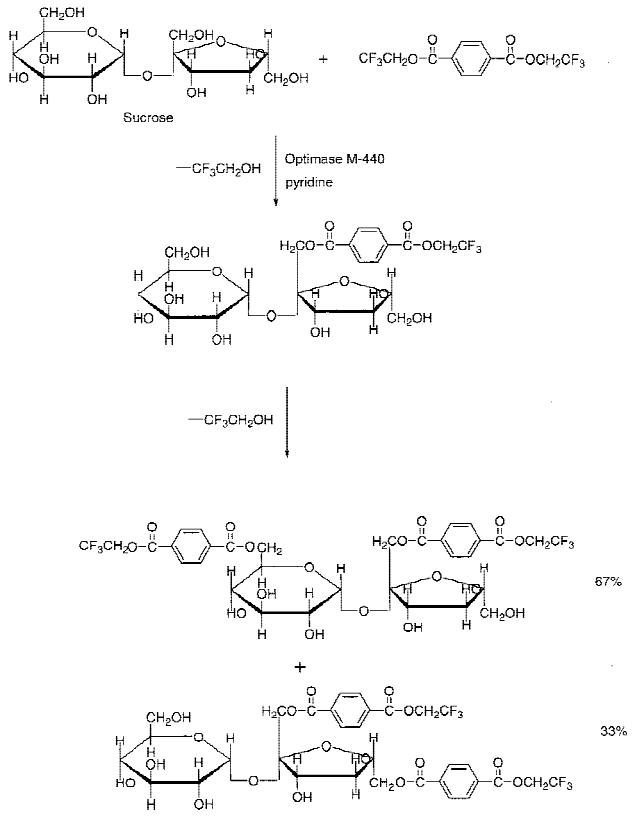


Figure 2. Scheme of sucrose acylation in anhydrous pyridine catalyzed by Optimase M-440.

Chemical Synthesis of Sucrose-Containing Aromatic Polymers

Polyester

Sucrose-containing aromatic polyester was prepared via solution polycondensation at 70°C for 24 h, as shown in Figure 3. The product was separated from the catalyst and unreacted diester by precipitation into acetone and dried at room temperature under vacuum for 24 h. A white powder was obtained in 65% yield. Without the zinc acetate catalyst, there was no formation of polymer as evidenced by TLC.

Structural analysis of the polymer was carried out using ^{13}C-NMR in DMSO with tetramethylsilane as internal stan-

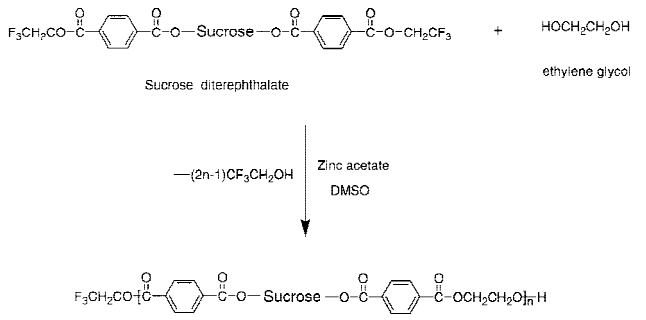


Figure 3. Chemoenzymatic synthesis of the sucrose-containing polyester.

dard. The representative ^{13}C-NMR is given in Table II. Clear downfield chemical shifts of carbons C1′, C6, and C6′ (data not shown) suggest that the ester bond between the benzene ring and the sucrose moiety was retained in the polyester. The regions of the NMR spectrum displaying the most significant changes from the starting diester were those lying between 60.3 and 61.2 and 120.2 and 126.8 ppm. These peaks are two quartets for the methylene carbon (—$\underline{C}H_2CF_3$) and trifluorocarbon (—$CH_2\underline{C}F_3$), respectively, in the trifluoroethyl group at the both end of the diester. These quartets were apparent in the spectrum of TFE-terephthalate, sucrose monoterephthalate, and sucrose diterephthalate. In the spectrum of the polymer, however, these peaks totally disappeared. The disappearance of these peaks for the trifluoroethyl group suggests that both ends were terminated by ethylene-glycol moiety.

To determine the number average molecular weight of the polyester, end-group analysis was performed using ^{13}C-NMR spectroscopy. The most informative peaks in the spectra are those for carbons in ethylene-glycol moiety — three singlets at δ 58.97, 63.22, and 67.00 ppm (Table II). They were assigned to the different ethylene carbons in the polymer. Singlets at 58.97 and 67.00 ppm were assigned to the α and β carbons, respectively, in the ethylene-glycol moiety at the end of the polymer and the areas of both absorptions were equal as expected. The singlet at 63.22 was for the ethylene carbons in the main chain of the polymer. The next most informative peaks in the spectra were those for carbonyl carbons — three singlets at δ 164.48, 164.94, and 165.05 ppm assigned to the emboldened and underlined carbons in Figure 4. The spectrum between 164 and 166 ppm arose from changes in the environment of the carbonyl carbons in the polyester. Singlets at 164.94 and 165.05 ppm, which were only partially resolved from each other, were assigned to the carbons in Figure 4a and b. One is for the carbonyl carbon between the sucrose moiety and the aromatic ring and the other was for that between the ethylene-glycol moiety and the aromatic ring. The areas of absorption at 164.94 and 165.05 appear visually to be equal, as expected, which confirms our suggested structure of the polymer. The singlet at 164.48 was for the carbonyl carbon adjacent to esterified oxygen from the ethylene-glycol moiety at both ends of the polymer. Measuring the peak intensities of these informative carbons, number average molecular weight was determined to be 3200 Da. Hence, on the basis of NMR data, nearly 5 sucrose, 10 TFE-terephthalate, and 5 ethylene-glycol molecules are joined together through ester linkages in the polyester and the polyester is a poly(ethylene-terephthalate) with sucrose contained in the polymer backbone.

Table II. The representative ^{13}C-NMR of the sucrose-containing polyester.

Carbon	δ (ppm)
α Carbon in the ethylene glycol moiety at the end of the polymer (—$CH_2\underline{C}H_2OH$)	58.97
β Carbon in the ethylene glycol moiety at the end of the polymer (—$\underline{C}H_2CH_2OH$)	67.00
Ethylene carbons in the polymer backbone (—C(O)O—$\underline{C}H_2\underline{C}H_2$—C(O)O—)	63.22
Carbons in sucrose moiety	Almost the same with sucrose diester (Table I)
Hydrogenated carbons in benzene ring	129.6 (singlet)
Para-substituted carbons in benzene ring	133.5 (doublet)
Carbonyl carbons in ester bonds	164.4–165.8

Polyamide

A sucrose-containing aromatic polyamide was also prepared via solution polycondensation at 70°C for 12 h as shown in Figure 5. The product was separated by precipitation into acetone and dried at room temperature under vacuum for 24 h. A white powder was obtained in 73% yield.

Structural analysis of the product was performed with ^{1}H- and ^{13}C-NMR in DMSO. The representative ^{1}H-NMR of the product is given in Table III. The most informative peak for the amide bond formation was the triplet centered at δ 8.85, which was absent in the spectra of both monomers, viz. sucrose diterephthalate and ethylene-diamine. The adsorption appeared as a broad and low hump far downfield, which represents characteristic adsorption by —CON**H**— protons of amides. The appearance of the peak in the spectrum apparently indicates that an amide bond was formed in the product. The peak for the amine protons at the end of the polymer appeared as a singlet at δ 1.34, which was almost the same position as that for amine protons of the starting monomer, ethylene-diamine (δ 1.33). The singlet at δ 3.02

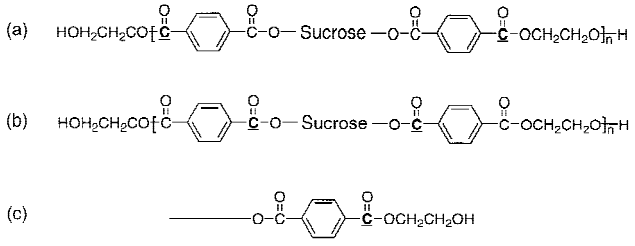


Figure 4. Carbonyl carbons in different environments in the sucrose-containing polyester.

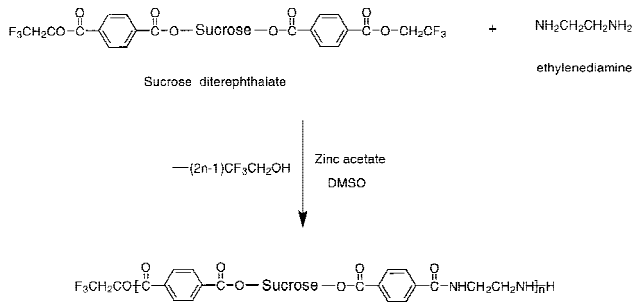


Figure 5. Chemoenzymatic synthesis of the sucrose-containing polyamide.

was assigned to protons of α carbon of amine, and the singlet at δ 3.49 was assigned to protons of β carbon of amine. The sizes of the peaks at δ 3.02 and 3.49 were equal, as would be expected by our assignments. The protons of the benzene ring showed a low-field adsorption characteristic of aromatic protons.

End-group analysis was applied to determine the number average molecular weight (M_n) of the polymer and the amine end-group was used. Molecular weight was estimated to be 4300 Da by using the amine end and amide or benzene ring areas. This calculation of degree of polymerization was based on the assumption that the end-groups are equally divided between amine, which was estimated directly from the area of the adsorption at δ 1.34 ppm, and the trifluoroethyl group of the sucrose diterephthalate moiety, which gave an adsorption near δ 5.0 ppm that appeared visually to be the same size but could not be integrated accurately due to interference from other adsorptions.

^{13}C-NMR was also carried out for polymer characterization. The shifts observed in the spectrum of the polyamide were almost the same when compared with those of sucrose diterephthalate. This indicates that the acylation of the C1′, C6, and C6′ hydroxyls was maintained in the polyamide backbone and no crosslinking appeared to be formed during the polymerization, and the polymer was a linear polymer formed by the reaction of ethylene-diamine with the external ester linkage of the sucrose diterephthalate.

The characterization of the polymer was also carried out using infrared spectroscopy, as shown in Figure 6, in which the adsorption bands characteristic of some groups are indicated. The spectra of the starting monomers, sucrose diterephthalate, and ethylene-diamine are also shown in Figure 6 for comparison. Ethylene-diamine showed N—H bending at 930 and 1600 cm^{-1} and N—H stretching at 3200 to 3500 cm^{-1}. In the spectrum of the sucrose diester, out-of-plane C—H bending of the aromatic ring gave an adsorption band at 727 cm^{-1} and ester adjacent to the aromatic ring showed a strong C = O stretch at 1730 cm^{-1}. The band for C—O stretching of secondary alcohols appeared at 1107 cm^{-1} and O—H stretching gave a strong, broad band st 3200 to 3500 cm^{-1}. These are due to the presence of the sucrose moiety. Another strong band due to C—F stretching, appears in the 1200 to 1300 cm^{-1} region.

Table III. The representative ^{1}H-NMR of the sucrose-containing polyamide.

Protons	δ (ppm)
Protons of amine at the end of the polymer: —$CH_2CH_2N\underline{\mathbf{H}}_2$	1.34
Protons of α carbon of amine: —$CH_2C\underline{\mathbf{H}}_2NH_2$	3.02
Protons of β carbon of amine: —$C\underline{\mathbf{H}}_2CH_2NH_2$	3.49
Protons of ethylene unit in the polymer backbone: —CONH—$C\underline{\mathbf{H}}^2C\underline{\mathbf{H}}_2$—NHCO—	1.34
Protons in benzene ring	7.96–8.11
Protons of amide bond in the polymer backbone: —CON$\underline{\mathbf{H}}$—	8.85

In the spectrum of the polyamide, the most conspicuous change was the appearance of two new bands at 1500 to 1700 cm^{-1}. The band at 1545 cm^{-1} was due to N—H bending (RCONHR′) and that at 1647 cm^{-1} was a C = O band in an amide, both of which strongly confirm the formation of an amide bond in the polymer. The bands due to the aromatic ring and sucrose moiety still appeared in the polymer spectrum. Hence, the structural analysis of the polymer as shown by infrared spectroscopy is consistent with the incorporation of sucrose into the polymer backbone.

The molecular weights of the polymers were somewhat lower than could be expected given a chemical polymerization, which may have been due to the unconventionally large-sized bulky monomer, sucrose diterephthalate. To obtain higher molecular weights, it is critical that equimolar mixtures of reactants are used, otherwise the yield of the high molecular weight polymer is low. This condition, however, is very difficult to achieve in the laboratory, particularly with this kind of multistep synthesized monomer. Another factor that tends to reduce the yield of long polymer molecules, even when equal numbers of functional groups are employed, is the equilibrium that occurs between the reactants and products during condensation reactions. If the concentration of the condensate, such as 2,2,2-trifluoroethanol in this case, is allowed to build up, the reaction may stop and a reverse reaction can even be forced. Thus, more precise control over the equimolarity and removal of the condensate may yield higher molecular weight polymers.

In this study, highly regioselective enzymatic synthesis and conventional polymerization were combined for the synthesis of two sucrose-containing aromatic polymers. Sugar-based polymers can be expected to find application in value-added products in the pharmaceutical industry (drug-delivery system) and aromaticity in the polymer backbone imparts a hydrophobic property in the sugar-based polymer that may be necessary and important depending on the area of application.

Broadening the application of chemoenzymatic synthesis routes requires: (i) the development of new enzymes as well thorough classical enzyme screening, such as by site-directed mutagenesis of enzymes with a known structure; and (ii) the development of chemoenzymatic methodologies for the synthesis of novel compounds. A combination of these two approaches will open the way toward production of a large set of new compounds with novel properties. The

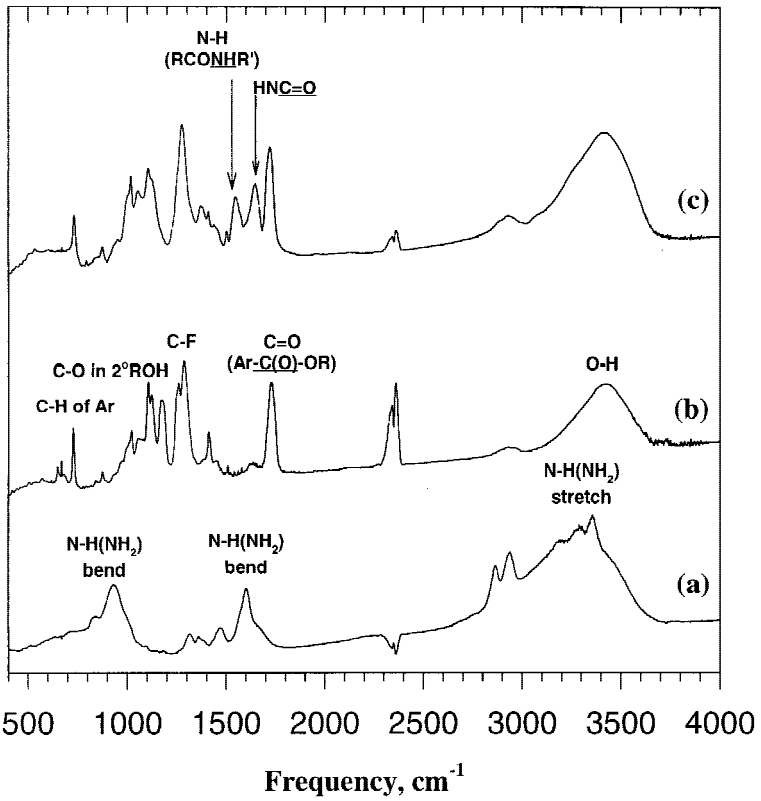


Figure 6. Infrared spectra of: (a) ethylene-diamine; (b) sucrose diterephthalate; and (c) sucrose-containing aromatic polyamide.

present work represents another advancement in the use of combined enzymatic and chemical synthesis in nonaqueous media.

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