# Advances in Polymer Science

# 121

S. Kobayashi, S. Shoda, H. Uyama
Enzymatic Polymerization and Oligomerization

K. E. Geckeler
Soluble Polymer Supports for Liquid-Phase Synthesis

K. Kishore, K. Ganesh
Polymers Containing Disulfide, Tetrasulfide, Diselenide and Ditelluride Linkages in the Main Chain

D. B. Priddy
Thermal Discoloration Chemistry of Styrene-co-Acrylonitrile

## Polymer Synthesis/ Polymer Engineering

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With contributions by
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# 121 Advances in Polymer Science

# Editors

# Table of Contents

# Enzymatic Polymerization and Oligomerization

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Polymerizations and oligomerizations catalyzed by various enzymes including hydrolases, transferases and peroxidases are reviewed. Optically active polyesters have efficiently been prepared by lipase-catalyzed asymmetric polymerizations using various dicarboxylic acid derivatives as monomers. A novel method for regio- and stereoselective synthesis of polysaccharides and oligosaccharides has been developed by enzymatic polycondensations of glycosyl fluoride monomers. The methodology has successfully been applied to the first in vitro synthesis of cellulose via a nonbiosynthetic path. A variety of polyaniline derivatives have been produced by peroxidase-catalyzed oxidation polymerizations of aniline derivatives in relation to their electronic properties.



Advances in Polymer Science, Vol. 121

## 1 Introduction

The progress of macromolecular science has been strongly attributed to the creation of a new class of polymers which are produced by the chemical modification of naturally occurring biopolymers or by the polymerization of various monomers having novel structures. All of the polymerization reactions utilized in polymer synthesis can be classified into the following four categories according to the chemical nature of the polymerization catalysts: radical polymerization [1], cationic polymerization [2], anionic polymerization [3], and coordination polymerization [4]. As the structures of targeting polymers are becoming more complicated, polymerization catalysts to promote the reaction under milder conditions and with higher regio- and stereoselectivities are required, and enormous efforts have been devoted to the development of new polymerization catalysts in order to achieve these requirements.

Enzymes have several remarkable catalytic properties compared with other types of catalyst in terms of the selectivity, high catalytic activity, lack of undesirable side-reactions, and operation under mild conditions. In addition, with the advent of genetic engineering, it will be possible to produce a wider range of enzymes on a larger scale expanding the number of enzymes available for synthetic reactions. Although many synthetic reactions catalyzed by enzymes have appeared in synthetic organic chemistry [5–10], few examples on polymerization reactions have been reported so far. This is probably due to the fact that the structural variation of our synthetic targets has only in recent years begun to make highly selective polymerizations necessary in response to the increasing demands for the production of various functional polymers in material science. The present review deals with recent advances in polymerization reactions catalyzed by an enzyme, proposing the term "enzymatic polymerization" as a novel concept in polymer synthesis.

In nature, all of the reactions producing important biopolymers are catalyzed by enzymes. It is, therefore, necessary to define clearly the term "enzymatic polymerization". There are generally three classes of biopolymer syntheses catalyzed by an enzyme.

1. Biosynthesis in vivo (in living cells) via biosynthetic pathways, e.g. naturally occurring reactions in all living systems.
2. Biosynthesis in vitro (outside cells) via biosynthetic pathways, e.g. a polymerization of a substrate of a phosphate derivative catalyzed by a polymerase enzyme in the cell-free extract.
3. Chemical synthesis in vitro (in test tubes) via non-biosynthetic pathways catalyzed by an isolated enzyme.

Now, we can define the above class (3) as "enzymatic polymerization". Biosyntheses via classes (1) and (2) produce naturally occurring biopolymers (macromolecules) in almost all cases. "Enzymatic polymerizations" of class (3), on the other hand, allow us to produce not only naturally occurring bio-

polymers but non-natural synthetic polymers, depending on the combination of substrate monomers and enzymes. The first chapter in this article deals with a brief review of in vivo polymerization reactions, namely biosynthesis of nucleic acids, proteins, polysaccharides, rubbers, and microbial polyesters, which belong to classes (1) and (2). The following chapters are concerned with the recently developed "enzymatic polymerization" catalyzed by hydrolases, transferases, and peroxidases, which belong to class (3).

## 2 A Brief Review of Polymerization Reactions via Biosynthetic Pathways

Biologically important macromolecules such as nucleic acids, proteins, and polysaccharides are not formed by direct condensation of the corresponding constructing units, nucleotides, amino acids, and monosaccharides, respectively. The activated precursors of phosphate derivatives formed by utilizing a high energy compound, namely adenosine triphosphate (ATP), are key monomeric substances for the synthesis of these macromolecules. The biosynthetic process therefore consists of the following two steps. The first step involves the formation of an activated monomer of phosphate ester derivative. The second step is the polymerization process catalyzed by an enzyme having high stereospecificity toward the substrate.

### *2.1 Nucleic Acids*

A typical example of enzymatic synthesis of nucleic acids is the transcription of the genetic code from DNA to messenger RNA (mRNA) [11]. The polymerization is catalyzed by RNA polymerase which exists in virtually all cells. This enzyme connects ribonucleotides by catalyzing the formation of the internucleotide 3′-5′ phosphodiester bonds. The monomers are triphosphates of adenine (A), cytosine (C), guanine (G), and uracil (U), whose nucleic acid base parts specifically pair with the corresponding bases of DNA, thymine (T), guanine (G), cytosine (C), and adenine (A), respectively. The polymerization takes place by releasing pyrophosphate (Fig. 1).

### *2.2 Proteins*

Protein synthesis begins in the cell nucleus with synthesis of mRNA. It acts as a template on the surface of the ribosomes by making a triplet pair with transfer RNA (tRNA) which carries amino acid residues, to the site of mRNA. Codon-anticodon pairing occurs between mRNA and tRNA, and an amide bond

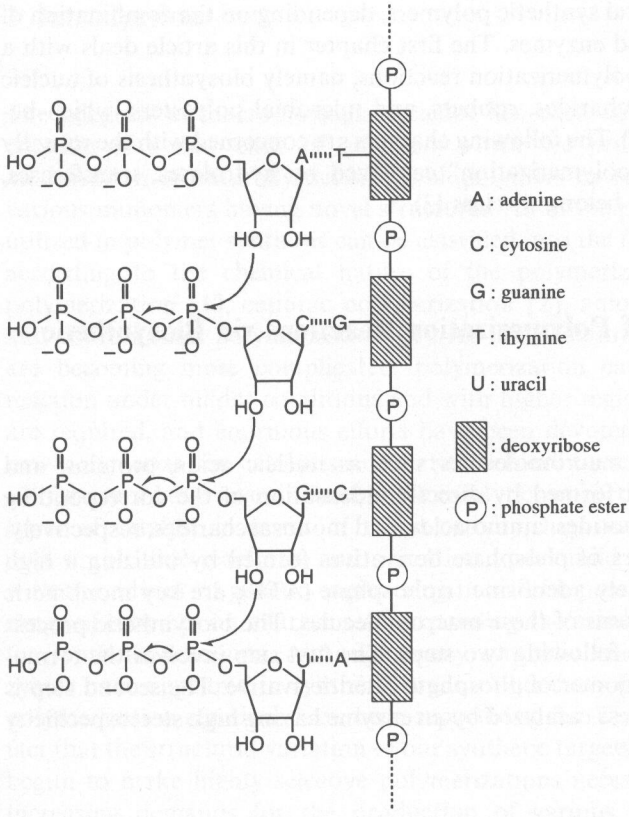



**Fig. 1.** Transcription of genetic code from DNA to messenger RNA (mRNA)

formation is achieved enzymatically (Fig. 2). After the first amide bond is formed the ribosome moves to the next codon on mRNA [12].

Concerning the activation of amino acids for constructing the peptide bond, the following process has been elucidated (Fig. 3). The first step is the formation of adenosine monophosphate of an amino acid (aminoacyl-AMP) by the reaction of an amino acid and adenosine triphosphate (ATP) catalyzed by aminoacyl-tRNA synthase (ARS). The resulting aminoacyl-AMP is further attacked by a hydroxyl group of a specific transfer RNA giving rise to an aminoacyl-tRNA as a precursor for the peptide bond formation.

## *2.3 Polysaccharides*

The typical naturally occurring polysaccharide, cellulose, is enzymatically synthesized in vivo starting from an activated form of glucose, uridine diphosphate

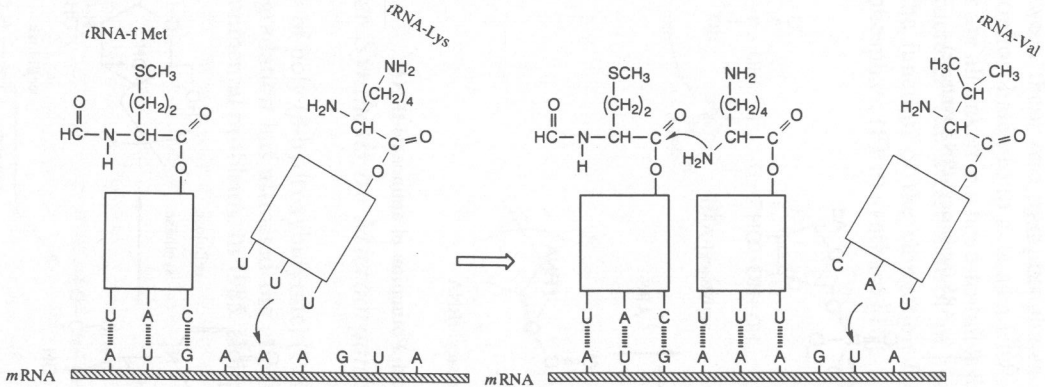



**Fig. 2.** Peptide bond formation via codon-anticodon pairing between mRNA and tRNA on ribosome

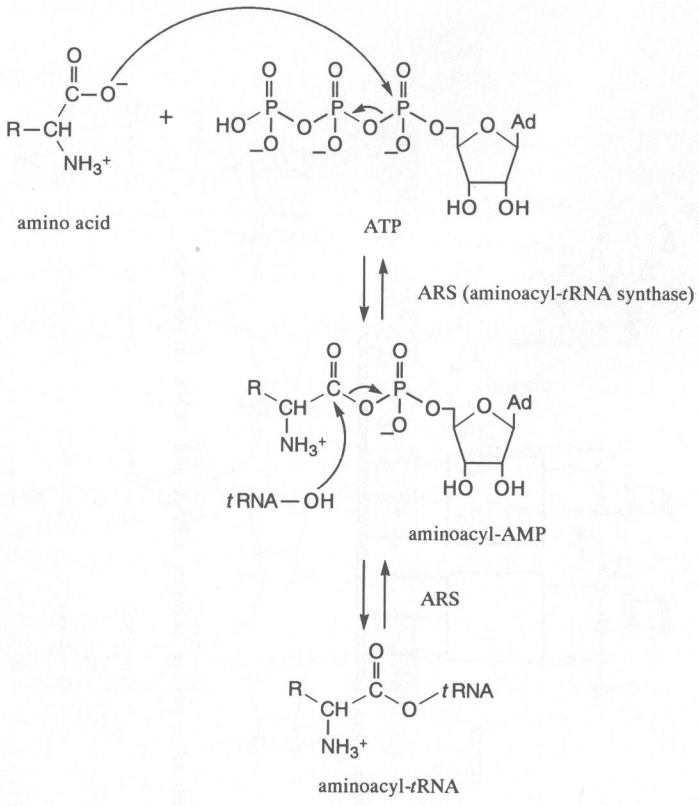
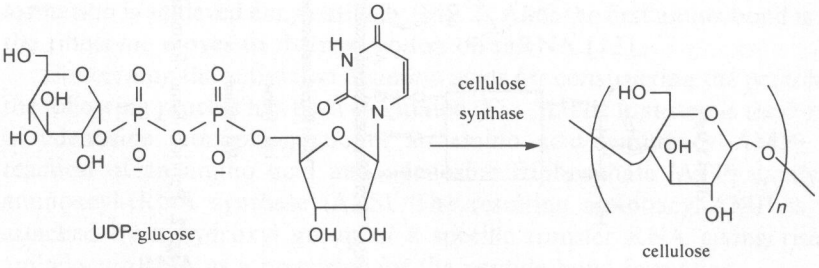


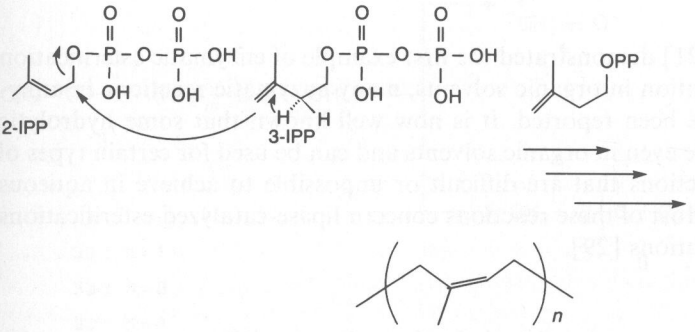

**Fig. 3.** Activation process of amino acid: Formation of aminoacyl-tRNA




glucose (UDP-glucose) catalyzed by cellulose synthase. Formation of cellulose according to a biosynthetic pathway using *Acetobacter xylinum* [13, 14] or *Phaseolus aureus* extracts [15] with a nucleoside diphosphate sugar (ADP-, CDP- GDP-, or UDP-glucose) as substrate has been reported.

## 2.4 Rubbers

Natural rubber can be regarded as a 1,4-addition polymer of isoprene. The basic building block of five carbons for the polymerization is 3-isopentenylpyrophosphate (3-IPP). The first reaction is an enzymatic isomerization of the olefin of 3-IPP to 2-isopentenylpyrophosphate (2-IPP). The carbon-carbon bond formation between these two pyrophosphates initiates the polymerization in which the pyrophosphate group acts as a leaving group. The isoprene units of natural rubber are all linked in a head-to-tail fashion and all of the double bonds have a *cis*-structure. The stereocontrol of the formation of the *cis*-unit is achieved by the function of the elongation factor which combines with the farnesyl pyrophosphate (FPP) synthase [16].




## 2.5 Polyester Synthesis by Microorganisms

The chemistry of poly ($\beta$-hydroxybutyrate) (PHB) involving structure, biosynthesis, and degradation has attracted the attention of scientists in connection with the environmental problems. In 1985, Holmes et al. at ICI published that a

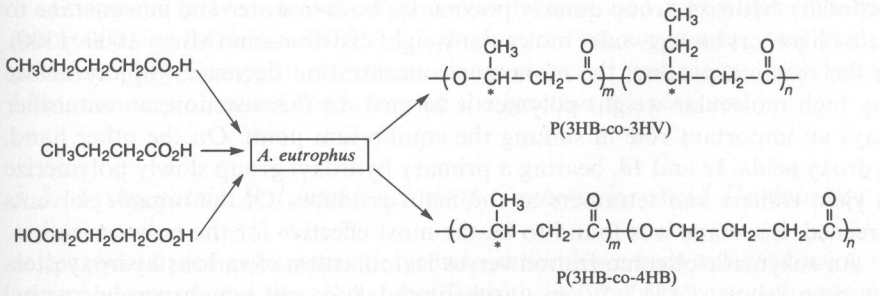



**Fig. 4.** Synthesis of random copolyester by *Alcaligenes eutrophus*

random copolymer P(3HB-*co*-3HV) of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) is produced by *Alcaligenes eutrophus* growing on glucose and propionic acid [17]. The ratio of 3HV can widely be controlled from 0 to 95 mol % by growing the microorganism in the presence of valeric acid and butyric acid [18]. The addition of 4-hydroxybutyric acid gave a new random copolyester having a 3HB unit and a 4-hydroxybutyrate (4HB) unit [19] (Fig. 4). Studies on the degradability of these poly(hydroxyalkanoates) by the action of a microorganism from the soil is extensively in progress in order to create new biodegradable polymers [20].

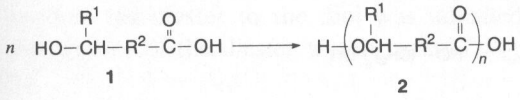
## 3 Polyester Synthesis by Enzyme Catalysts

Since Klibanov [21] demonstrated the first example of enzymatic esterification and transesterification in organic solvents, many enzymatic reactions in anhydrous media have been reported. It is now well known that some hydrolytic enzymes are stable even in organic solvents and can be used for certain types of condensation reactions that are difficult or impossible to achieve in aqueous media [22–28]. Most of these reactions concern lipase-catalyzed esterifications and transesterifications [29].

### *3.1 Polymerization of Achiral Carboxylic Acid Derivatives*

The first paper describing an enzymatic synthesis of polyesters appeared in 1986, where achiral hydroxy acids were used as monomers [30]. The polycondensation of 12-hydroxyoctadecanoic acid **1a**, 12-hydroxy-*cis*-9-octadecenoic acid **1b**, 16-hydroxyhexadecanoic acid **1c** and 12-hydroxydodecanoic acid **1d** catalyzed by *Candida rugosa* lipase and *Chromobacterium viscosum* lipase afforded the corresponding polyesters **2** having a long alkyl chain. The reaction was carried out in water or in a non-polar organic solvent, and the molecular weight ($M_n$) of the resulting polymers was 600–1300. Monomers **1a** and **1b** bearing a secondary hydroxy group quickly polymerize both in water and in isooctane to yield oligomers having wider molecular weight distributions (Mn = 1000–1300). In the reaction process, the monomer concentration decreases rapidly before any high molecular weight polymer is formed. In this reaction, an emulsifier plays an important role in shifting the equilibrium point. On the other hand, hydroxy acids, **1c** and **1d**, bearing a primary hydroxyl group slowly polymerize to yield trimers and tetramers as the main products. Of the organic solvents screened, isooctane was found to be the most effective for the polymerization.

An enzymatic oligomerization versus lactonization of various hydroxyesters has been reported [31]. When unsubstituted $\beta$, $\delta$, and $\varepsilon$-hydroxyacid methyl esters **3** are subjected to the action of Porcine pancreatic lipase in anhydrous



**1**

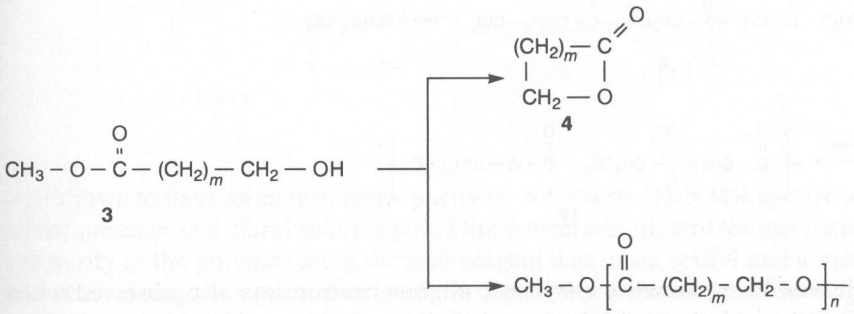
**2**

**1a** : $R^1 = (CH_2)_5CH_3$, $R^2 = -(CH_2)_{10}-$

**1b** : $R^1 = (CH_2)_5CH_3$, $R^2 = -CH_2-CH=^{cis}CH-(CH_2)_7-$

**1c** : $R^1 = H$, $R^2 = -(CH_2)_{14}-$

**1d** : $R^1 = H$, $R^2 = -(CH_2)_{10}-$



**3**

**4**

**5**

**3a** : $m = 1$

**3b** : $m = 3$

**3c** : $m = 4$

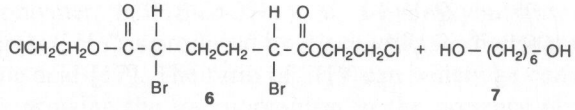
organic solvents, they exclusively undergo intermolecular transesterification to afford the corresponding oligomers **5**; lactonized product **4** was not obtained. In contrast, the substituted $\delta$-methyl-$\delta$-hydroxyester undergoes lactonization even in concentrated solutions.

Lipase-catalyzed polymerization between an achiral dicarboxylic acid and a primary glycol in an aqueous-organic mixture was reported [32]. A polycondensation of achiral 10-hydroxydecanoic acid catalyzed by lipase in benzene has also been claimed, however no convincing evidence was presented [33].

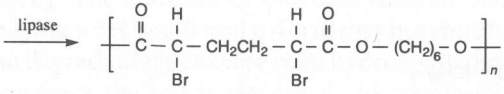
Enzymatic polycondensation of a dicarboxylic acid and a diol has been reported using lipase from *Candida cylindracea*, *Pseudomonas* sp., and Porcine pancreas [34]. Competition between linear polyester and macrocyclic lactone formation was observed depending on the reaction temperature.

## *3.2 Asymmetric Polymerization of Carboxylic Acid Derivatives*

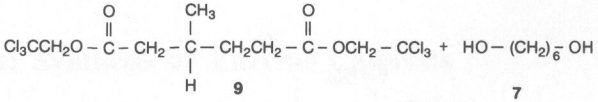
A lipase-catalyzed asymmetric polycondensation have been achieved using the racemic diester **6** and the achiral diol **7** (or the achiral diester and the racemic diol) in an organic solvent [35]. Optically active trimers and pentamers **8** were



6 7



8



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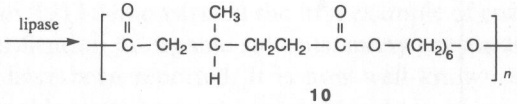
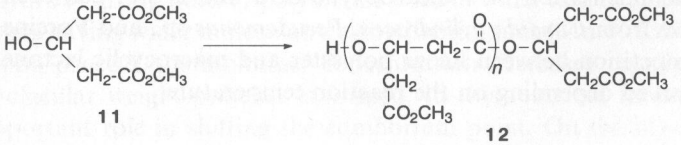


10

produced. An asymmetric enzymatic oligomerization was also observed when bis (2, 2, 2-trichloroethyl) ( ± )-3-methyladipate **9**, was used where the asymmetric carbon is farther from the reaction site than in **6**.

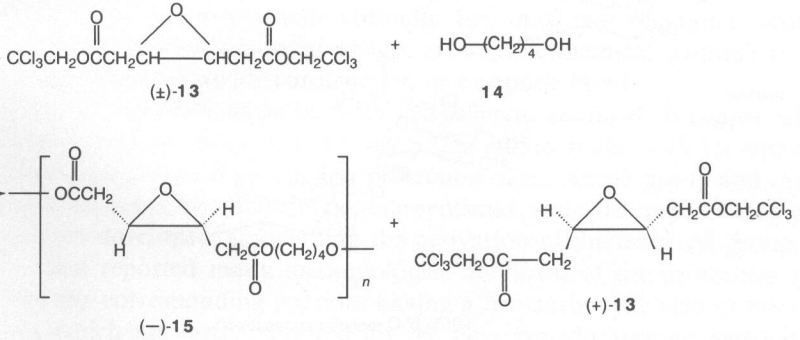
An enantioconvergent polymerization of symmetrical hydroxy diester **11** has been developed [36]. The polymerization utilizes the prochiral stereospecificity of enzymes, i.e. their ability to discriminate between enantiotopic groups of a prochiral molecule, in organic solvents. This approach is significantly potential for the asymmetric synthesis of chiral polyesters from achiral monomers possessing $\sigma$-symmetry. The enantiomer excess (ee) values (30–37%) of the resulting oligomer **12** are considerably higher than the reported values which had been obtained by a chemical polymerization of prochiral monomers with asymmetric catalysts [37].



11 12

Synthesis of an optically active epoxy-substituted polyester **15** by lipase-catalyzed polymerization has been demonstrated [38]. A highly enantioselective polymerization of a chiral, epoxide-substituted diester **13** with 1,4-butanediol **14** was performed using Porcine pancreatic lipase (PPL) as catalyst. The molar

ratio of the diester to the diol was adjusted to 2:1 assuming that only one enantiomer of the diester would react. The unchanged ( + )-monomer ( + )**13**




was shown to have an enantiomeric purity of > 95% by $^1$H-NMR spectroscopy in the presence of a chiral shift reagent. Direct determination of the stereochemical purity of the polymer using the shift reagent was unsuccessful and a method for determining the optical purity of the copolymer remains to be developed.

Transesterification of diphenyl carbonates with glycols proceeds using *Candida cylindracea* lipase of Porcine liver esterase as catalyst to give the corresponding polycarbonates [39]. Enantiomeric selectivity in transesterification of diphenyl carbonate with racemic 2-butanol was observed ( > 80% ee). Transesterification activity of diphenyl carbonate in a water-saturated organic solvent is strongly affected by the choice of solvent. The stability of the enzyme in dry conditions was also investigated. It is speculated that a small amount of water remains tightly bonded to the enzyme allowing retention of native conformation and hence the enzyme activity is held.

## *3.3 Sugar Containing Polyester*

Another advantageous feature of enzymes in an esterification reaction lies in their ability to distinguish a specific hydroxyl group of a complex polyalcohol like sugar derivatives. Klibanov et al. found that *Bacillus subtilis* protease (subtilisin) catalyzes the regioselective acylation of disaccharides **16**, as well as nucleosides and related compounds in *N*,*N*-dimethylformamide [40].

Sucrose-containing linear polyesters **19** have been prepared using various enzymes from sucrose **17** and dicarboxylic acid diesters **18** [41]. The polycondensation reaction proceeds in anhydrous pyridine. An alkaline protease from a *Bacillus* sp. catalyzes the esterification of sucrose with bis(2,2,2-trifluoroethyladipate) to give a sucrose-containing polyester with $M_w = 2100$. The sucrose polyester is highly water soluble and soluble in polar organic solvents.