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Z. Gomurashvili[a]; H. R. Kricheldorf[b]; R. Katsarava[a]
[a] Research Center for Medical Polymers and Biomaterials of Ministry of Health Care of Georgia and Georgian Technical University, Tbilisi-79, Rep. of Georgia [b] Institut fur Technische und Makromoleculare Chemie, Universitat Hamburg, Hamburg 13, Germany

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AMINO ACID BASED BIOANALOGOUS POLYMERS. SYNTHESIS AND STUDY OF NEW POLY(ESTER AMIDE)S COMPOSED OF HYDROPHOBIC α-AMINO ACIDS AND DIANHYDROHEXITOLES

Z. Gomurashvili,‡ H. R. Kricheldorf,†* and R. Katsarava‡

‡ Research Center for Medical Polymers
and Biomaterials of Ministry of Health Care of Georgia
and Georgian Technical University
PO Box 24, 380079 Tbilisi-79, Rep. of Georgia

† Institut fur Technische und Makromoleculare Chemie
Universitat Hamburg
Bundesstrasse 45, D-20146
Hamburg 13, Germany

Key Words: α-Amino Acid Esters, Sequential Poly(ester-amide)s, Isosorbide, Enzymatic Degradation

ABSTRACT

A new class of biodegradable poly(ester-amide)s was prepared by a two step method. At first isosorbide or isomannide were esterified with α-amino acids in the presence of *p*-toluenesulfonic acid, and the resulting esters bisammonium tosylates were isolated. Second, the amino groups were liberated and polycondensed with *p*-nitrophenyl esters of aliphatic dicarboxylic acids. The resulting poly(ester-amide)s were characterized by elemental analyses, viscosity and GPC measurements, by NMR spec-

* Author to whom correspondence should be addressed.

troscopy and DSC measurements. They proved to be materials having glass-transition temperatures in the range of 60-120°C. Using chymotrypsin or lipase, preliminary studies of the enzymatic degradation were performed.

INTRODUCTION

Amino acid based bioanalogous polymers (**AABBPs**) - regular poly(ester amide)s (**PEAs**) [1, 2] and poly(ester urethane)s (**PEURs**) [3] entirely composed of nontoxic building blocks like hydrophobic α-amino acids (most of which are an essential type), aliphatic dicarboxylic acids and diols proved to be promising biodegradable materials for various biomedical applications. These polymers, prepared in a simple way by solution polycondensation (**PC**) of di-*p*-toluenesulfonic acid salts of bis-(α-amino acid) α,ω-alkylene diesters with active diesters of dicarboxylic acids (**PEAs**) or active bis-carbonates of diols (**PEURs**), showed a wide range of material properties from fiber- and film-firming to hydrophilic elastomers and subjected specific or nonspecific hydrolysis with rather high rates depending mostly on the nature of α-amino acid used and on linking bonds in the backbones (i. e., class of polymer).

In the present paper, we have decided to use dianhydrohexitols (**DAH**) – 1,4:3,6-dianhydrosorbitol (**DAS**) and 1,4:3,6-dianhydromannitol (**DAM**) as diol components for preparing **AABBP** type **PEAs** and **PEURs** for the following reasons: Firstly, these monomers (in particular **DAS**) are available in industrial quantities and are derived entirely from renewable resources (starch), and represent a highly interesting diol components for the synthesis of heterochain polymers like polyesters [4-9], poly(ester-anhydride)s [10], polyethers [7], polyurethanes [6, 11], polycarbonates [6] and related polymers. Secondly, these compounds are used in pharmacy, consequently, they are nontoxic. Thirdly, we expected that the incorporation of rigid bicyclic fragments into the polymeric backbones would increase T_g of **AABBPs** (e.g. polyesters based on **DAH** exhibit a rather high glass transition temperature, T_g [5]). Fourthly, the secondary hydroxy groups in **DAH** are sterically rather hindered and relatively less active, and in **DAS** even unequal, and we hoped that the preparation of O,O'-bis-α-aminoacyl derivatives of **DAH** would lead to the remote from bicyclic fragments and sterically unhindered (by these fragments at least), highly active functional amino groups with equal activity that would facilitate the polymer synthesis from **DAH**. And finally, it was interesting to study the influence of these rigid bicyclic

fragments on the tendency of polymers to undergo enzyme catalyzed *in vitro* biodegradation as compared with analogous samples prepared from normal alkylenediols. To the best of our knowledge, no attempt has been made to apply these compounds for preparing biodegradable polymers having potential for biomedical applications.

In the present work, we have investigated: a) synthesis of monomers **1** - *p*-toluenesulfonic acid salts of O,O'-bis-(α–aminoacyl)-1,4:3,6-dianhydrosorbitol and O,O'-bis-(α–amino acyl)-1,4:3,6-dianhydromannitol, b) synthesis and characterization of regular **PEAs 3** by polycondensation of **1** with active bis(*p*-nitrophenyl) esters of aliphatic dicarboxylic acids **2,** and c) enzyme catalyzed *in vitro* hydrolysis of **PEAs 3.**

EXPERIMENTAL

Materials

All the solvents, pyridine (Py) and triethylamine (NEt_3) were purified by conventional methods.

α-Amino acids – L-phenylalanine (Phe), L-leucine (Leu), L-isoleucine (Ile), and L-methionine (Met) (Sigma), *p*-toluenesulfonic acid monohydrate, *p*-nitrophenol (Aldrich), **DAS** and **DAM** (Aldrich) were used without further purification.

Synthesis of p-Toluenesulfonic Acid Salts of O,O'-bis(L-α-aminoacyl) DAH, 1

*Synthesis of **1-DAS** (General Procedure)*

A suspension of an α-amino acid (0.1 mol), *p*-toluenesulfonic acid monohydrate (0.11 mol) and diol (0.05 mol) in 150 mL of toluene were heated and reflux with stirring. Heating was continued up to the evolution of 3.6 ml (0.2 mol) of water (≈ 12-18 hours). The heterogenous reaction mixture was cooled to room temperature and the solid products was filtered off, washed with toluene and dried under reduced pressure.

Synthesis of **1-DAM** has been carried out according to the same procedure using benzene as a reaction medium instead of toluene.

Yields: **1-DAS,Phe** 95%, **1-DAS,Leu** 88%, **1-DAS,Ile** 75%, **1-DAS,Met** 70%, **1-DAM,Phe** 90%.

Melting points after recrystallization from methanol/toluene mixture: **1-DAS,Phe** 264-266°C, **1-DAS,Leu** 253-256°C, 1290°C (decomposed), **1-DAS,Met** 210°C, **1-DAM,Phe** 253-255°C.

Specific rotations $[\alpha]_D$ in deg.dm^{-1}.g^{-1}.cm^3 (DMA, c = 1 g/dL, l = 1dm): **1-DAS,Phe** +70°, **1-DAS,Leu** + 41°, **1-DAS,Ile** + 46°, **1-DAS,Met** + 40°, **1-DAM,Phe** +115°.

Elemental analyses and IR data:

1-DAS,Phe: $C_{38}H_{44}N_2O_{12}S_2$ (784.90) Calcd.: C 58.15, H 5.65, N 3.57, S 8.17.
Found: C 57.96, H 5.51, N 3.49, S 8.28.
IR (KBr): 1160 cm^{-1} (-O-), 1735 cm^{-1} (-CO-).

1-DAS,Leu: $C_{32}H_{48}N_2O_{12}S_2$ (716.87). Calcd.: C 53.62, H 6.75, N 3.91, S 8.95.
Found: C 53.74, H 6.78, N 4.01, S 9.18.
IR (KBr): 1160 cm^{-1} (-O-), 1755 cm^{-1} (-CO-).

1-DAS,Ile: $C_{32}H_{48}N_2O_{12}S_2$ (716.87) Calcd.: C 53.62, H 6.75, N 3.91, S 8.95.
Found: C 53.71, H 6.81, N 3.98, S 9.22.
IR (KBr): 1155 cm^{-1} (-O-), 1752 cm^{-1} (-CO-).

1-DAS,Met: $C_{30}H_{44}N_2O_{12}S_2$ (752.94) Calcd.: C 47.86, H 5.86, N 3.72, S 17.03
Found: C 47.91, H 6.02, N 3.77, S 17.24
IR (KBr): 1181 cm^{-1} (-O-), 1761 cm^{-1} (-CO-).

1-DAM,Phe: $C_{38}H_{44}N_2O_{12}S_2$ (784.90) Calcd.: C 58.15, H 5.65, N 3.57, S 8.17
Found: C 58.06, H 5.56, N 3.45, S 8.28
IR (KBr): 1176 cm^{-1} (-O-), 1756 cm^{-1} (-CO-).

Synthesis of Bis(p-nitrophenyl) Esters of Diacids 2 (General Procedure)

To the chilled solution of 28.0 g (~ 0.201 mol) of *p*-nitrophenol and 16.3 mL (~ 0.201 mol) of dry pyridine in 200 mL of ethylacetate a solution of diacid dichloride (0.1 mol) in 100 mL of ethylacetate was added dropwise over 30 minutes. Afterwards, the reaction mixture was heated up to r.t. and stirred for 2 hours. Ethylacetate was evaporated, the obtained solid product was dried, washed with acidified water (pH 2-3), distilled water and dried. Yields, 92-97%. After recrystallization from ethylacetate they have melting points: **2(4)** 123-124°C (lit. m.p. 123°C [10]), **2(6)** 108-110°C (lit. m.p. 110°C [10]), **2(8)** 104-105°C (lit. m.p. 103°C [10]). M.p. of diester **2(10)** 106-108°C, elemental analyses for **2(10):** $C_{24}H_{28}N_2O_8$:

Calcd.:	C 61.01	H 5.97	N 5.9
Found:	*C 61.24*	*H 6.02*	*N 5.90*

Synthesis of PEAs 3 (General Procedure)

To the stirred mixture of 6 mmol of **1** and 6 mmol of **2** in 4.12 mL of DMA 0.88 mL (6.3 mmol) of NEt_3 (total volume 5.0 mL, c = 1.2 mol/L) was added under dry nitrogen and heated at 65°C for 48 hours. In all cases, the reaction proceeded homogeneously. The obtained viscous reaction solution was poured into iced water, the precipitated product was filtered off and thoroughly washed with water. The obtained solid products were dried at 40°C *in vacuo*. The dried polymer was dissolved in chloroform and reprecipitated in ethyl acetate, filtered and dried at room temperature *in vacuo*. The yields and η_{inh} values of **PEAs 3** are given in Table 1.

Measurements and Techniques

The η_{inh} values were measured with an automated Ubbelohde viscometer at 20°C. The IR spectra were recorded from KBr pallets (monomers) or as films (polymers) cast on NaCl plates with Nicolet SXB - 20 FT-IR spectrometer. The 100 MHz ^{1}H NMR spectra (5 mm o.d. sample tubes) and 25,4 MHz ^{13}C NMR spectra (10 mm o.d. sample tubes) were obtained on a Bruker AC-100 FT spectrometer using solutions in $CDCl_3$ containing tetramethylsilane (TMS). M_w and

TABLE 1. **PEAs 3** Obtained by Polycondensation of **1** with **2** According to Scheme 3[a)]

#	PEA 3	Yield in %	η_{inh} [b)] dL / g	Mw [c)]	Mn [c)]	Mw/Mn	Tg [d)] °C
1	**3-DAS,Phe(4)**	85	0.19	15000	13000	1.0	96
2	**3-DAS,Phe(6)**	88	0.32	21500	14000	1.5	91
3	**3-DAS,Phe(8)**	91	0.38	37000	24000	1.5	78
4	**3-DAS,Phe(10)**	95	0.46	47000	27000	1.7	76
5	**3-DAS,Leu(4)**	65	0.12	9000	7000	1.4	91
6	**3-DAS,Leu(8)**	72	0.20	27000	16000	1.7	89
7	**3-DAS,Ile(4)**	68	0.17	16000	10000	1.6	102
8	**3-DAS,Ile(8)**	71	0.12	insol	-	-	91
9	**3-DAS,Met(4)**	61	0.14	insol	-	-	71
10	**3-DAS,Met(8)**	64	0.13	10000	6000	1.7	61
11	**3-DAM,Phe(4)**	91	0.22	insol	-	-	99
12	**3-DAM,Phe(8)**	96	0.48	50000	32000	1.6	70

a) Polycondensation in DMA at 65°C, duration 48 h. Concentration of each monomer 1,2 mol/L.
b) Measured in $CHCl_3$ at 20°C with c = 2 g / L.
c) Weight- and number-averge molecular weights, determined by GPC, eluent tetrahydrofuran.
d) From DSC measurements with a heating rate of 20°C/min (first heating).

M_n were determined by GPC using a Kontron HPLC-420 instrument equipped with a Waters differential refractometer (Model 410); GPC was carried out in tetrahydrofuran using polystyrene standards.

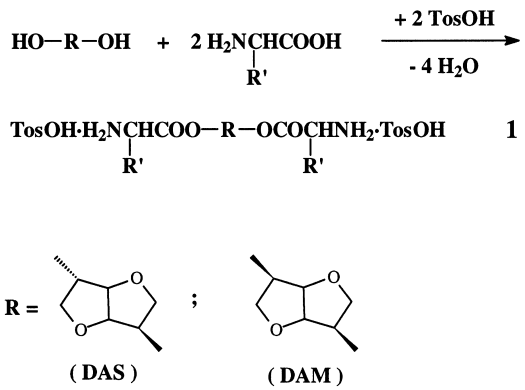
The DSC measurements were made with a Perkin-Elmer DSC-7 at a heating rate of 20°C/min in Al pans under N_2. Specific rotations $[\alpha]_D$ in units $deg \cdot dm^{-1} \cdot g^{-1} \cdot cm^3$ were measured on a polarimeter Perkin-Elmer 241 with cell length $l = 1dm$.

In vitro hydrolysis measurements were carried out at 37°C and pH 7.4 in 5 mL 0.1 N NaCl solution using a "Radiometer RTS 822" titrator as described earlier [1]. The duration of each experiment - 70 minutes 0.1 N NaOH was used for titration. Films for *in vitro* hydrolysis study ($m \sim 200$ mg, $D = 5$ cm) were cast from chloroform solutions and dried at 65°C up to constant weights.

RESULTS AND DISCUSSION

Monomer Syntheses

The key monomers, the di-*p*-toluenesulfonic acid salts of O,O'-bis-(α–aminoacyl)-1,4:3,6 dianhydrohexytols **1**, were prepared according to Scheme 1, using a method, described earlier [1-3]. The interaction of **DAM,** having two



R' = $CH_2C_6H_5$ (Phe), $CH_2CH(CH_3)_2$ (Leu), $CH(CH_3)CH_2CH_3$ (Ile), $(CH_2)_2SCH_3$ (Met)

L-α-amino acids are used for the synthesis of 1.

Designations of 1: e.g. 1-DAS,Phe means: di-*p*-toluenesulfonic acid salt 1 based on DAS and L-phenylalanine, etc.

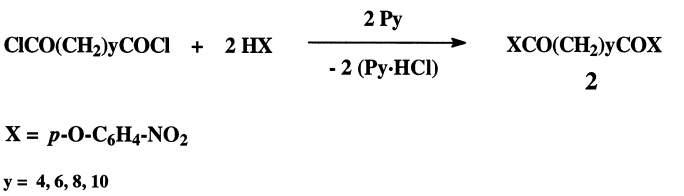
Scheme 1.

equivalent and relatively less hindered endo-hydroxyl groups, with α-amino acid proceeded smoothly in refluxing benzene, and two moles of water was liberated per one mole of diol after 10-12 hours. However, in the case of **DAS,** having two non-equivalent hydroxyl groups (endo-5 and exo-2), only one mole of water was liberated per one mole of diol after prolonged (48 hours) heating. This means that less active an exo-hydroxyl group did not take part in the condensation reaction under the reaction conditions used. And, only at a higher temperature – in refluxed toluene, was the reaction completed and compounds **1-DAS** were obtained.

In this study, five different types of new di-*p*-toluensulfonic acid salts (of the general formula **1-DAS,Phe, 1-DAS,Leu, 1-DAS,Ile, 1-DAS,Met** and **1-DAM,Phe**) were synthesized from the four α-amino acids and two *bis*-secondary diols. The essential characteristics of these diamino-diester monomers are given in the Experimental and in Table 1. The structures of the monomers of structure **1** were confirmed by both elemental analysis and IR-spectra. All of the diamino-diester monomers were optically active. They had melting temperatures ranging from 210 to 266°C. The yields of the monomer synthesis ranged from 70 to 95%.

Di-*p*-toluenesulfonic acid salt monomers **1** are relatively inexpensive monomers and can be obtained in a high yield based on our method. The monomers can easily be purified by recrystallization from toluene-methanol mixture. These compounds contain two ester groups which can undergo either specific or non-specific hydrolysis. They are composed entirely of non-toxic building blocks and have the potential to serve as starting materials for the synthesis of various new biomaterials.

The active diesters **2** were prepared in a nearly quantitative yield by a well-known reaction, between acids dichlorides (1 mol) and *p*-nitrophenol (2 mol) in the presence of pyridine (2 mol) in organic media (e.g. ethylacetate), according to Scheme 2. These compounds were characterized by melting points, elemental analysis and IR-data.



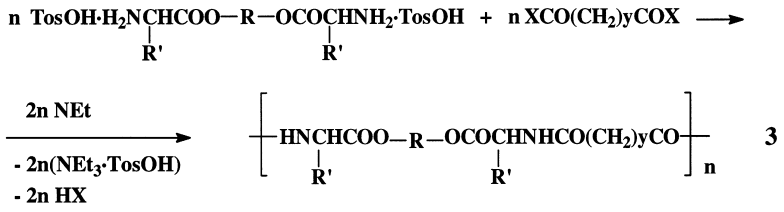
Designations of diesters 2: e.g. 2(4) means: di-*p*-nitrophhenyl adipate, etc.

Scheme 2.

Polymer Syntheses and Characterization

The **PEAs 3** were synthesized by solution polycondensation of the salts **1** with active diesters **2** in the presence of a little excess of NEt_3 (2,2 mol NEt_3 per 1,0 mol of **1**) according to Scheme 3. For the polycondensation of **1** with **2** we used reaction conditions which have been found to be optimum for the polycondensation of salts like **1** with **2(4)** [2]: reaction temperature 65°C, duration 48 h, concentration of each monomer 1,2 mol/L, and DMA as the reaction medium. Under these conditions the reaction proceeds homogeneously, resulting in high-molecular-weight **PEAs 3,** in many cases ($\eta_{red.} \leq$ 0.48 dL/g, Table 1). In the case of the salt **1-DAS,Met,** the polycondensation was carried out under argon otherwise the gelation of the reaction solution was observed. This gel-formation obviously is connected with the oxidation reaction in which the sulfur atom of the **Met** takes part. The oxidation reaction proves that **3-DAS,Met(*y*)** had lost the solubility in organic solvents when precipitated in water, and had retained solubility when precipitated in water, containing cystein which prevented the sulfur atom from oxidation. Furthermore, **3-DAS,Met(*y*)** lost solubility when stored under atmospheric conditions. It should be noted that we did not observe this phenomenon previously [2], when α,ω-alkylene diols were used instead of **DAHs.**

The structure of **PEAs 3** was confirmed by elemental analysis (Table 2) and IR-spectra (films cast on NaCl plates from chloroform solutions): carbonyl bands at 1660-1680 cm^{-1} (amide) and 1725-1740 cm^{-1}, and NH-vibrations at 3350 cm^{-1} which are typical for all **PEAs** of similar worth. ^{13}C NMR spectra recorded for **3-DAS,Phe(8)** (Figure 1) and **3-DAM,Phe(8)** confirmed the



For R and R' see Scheme 1, for X and *y* see Scheme 2.

Designation of PEAs 3: e.g. 3-DAS,Phe(4) means: PEA based on DAS, L-phenylalanine and adipic acid, etc.

Scheme 3.

TABLE 2. Specific Rotations and Elemental Analysis of **PEA 3**

#	PEA 3	$[\alpha]_D^{20}$ a)	Empirical formula (MW)	Elemental analyses	C	H	N	S
1	**3-DAS,Phe(4)**	+46	$C_{30}H_{34}N_2O_8$ $(550.60)_n$	Calcd	65.44	6.22	5.09	-
				Found	65.35	6.32	4.91	-
2	**3-DAS,Phe(6)**	+65	$C_{32}H_{38}N_2O_8$ $(578.66)_n$	Calcd	66.42	6.62	4.84	-
				Found	66.34	6.71	4.76	-
3	**3-DAS,Phe(8)**	+72	$C_{34}H_{42}N_2O_8$ $(606.71)_n$	Calcd	67.31	6.98	4.62	-
				Found	67.22	7.12	4.51	-
4	**3-DAS,Phe(10)**	+74	$C_{36}H_{46}N_2O_8$ $(634.76)_n$	Calcd	68.12	7.30	4.41	-
				Found	68.00	7.19	4.36	-
5	**3-DAS,Leu(4)**	+26	$C_{24}H_{38}N_2O_8$ $(482.57)_n$	Calcd	59.74	7.94	5.81	-
				Found	59.63	7.85	5.66	-
6	**3-DAS,Leu(8)**	+26	$C_{28}H_{46}N_2O_8$ $(538.68)_n$	Calcd	62.43	8.61	5.20	-
				Found	62.27	8.75	5.11	-
7	**3-DAS,Ile(4)**	-29	$C_{24}H_{38}N_2O_8$ $(482.57)_n$	Calcd	59.74	7.94	5.81	-
				Found	59.61	7.77	5.71	-
8	**3-DAS,Ile(8)**	-11	$C_{28}H_{46}N_2O_8$ $(538.68)_n$	Calcd	62.43	8.61	5.20	-
				Found	62.26	8.69	5.15	-
9	**3-DAS,Met(4)**		$C_{22}H_{34}N_2O_8S_2$ $(518.64)_n$	Calcd	50.95	6.61	5.40	12.36
				Found	51.16	6.87	5.59	12.08
10	**3-DAS,Met(8)**	+41	$C_{26}H_{42}N_2O_8S_2$ $(574.74)_n$	Calcd	54.33	7.34	4.87	11.16
				Found	54.54	7.43	5.05	10.97
11	**3-DAM,Phe(4)**		$C_{30}H_{34}N_2O_8$ $(550.60)_n$	Calcd	65.44	6.22	5.09	-
				Found	65.61	6.41	5.22	-
12	**3-DAM,Phe(8)**	+4.6*)	$C_{32}H_{38}N_2O_8$ $(578.66)_n$	Calcd	67.31	6.98	4.62	-
				Found	67.54	7.16	4.74	-

a) Optical rotation in $CHCl_3$, measured at 25°C , c= 1,0 g/dL (deg.dm^{-1}.g^{-1}.cm^3). Measured with a Perkin Elmer 241 polarimeter at a wavelenght of 578 nm, *) at wavelength of 589 nm.

assumed structure of **PEAs 3**. In the ^{13}C NMR spectrum of **3-DAS,Phe(8)**, four CO signals were observed (CO ester and CO amide are split), as it was expected taking into account the non-symmetrical structure of the diol**. PEAs 3** based on **DAS** are regular in respect to the amide and ester groups alternations, but stereo-irregular, because of different kinds of connection with two adjacent **DAS** moieties ($2 \rightarrow 2$, $2 \rightarrow 5$ and $5 \rightarrow 5$), as it was shown in ref. [4, 5] for polyesters based on **DAS** and aromatic diacids. In the case of **PEAs** based on **DAM** (recorded for **3-DAM,Phe(4)**, no splitting of CO-ester and CO-amide signals were observed in the ^{13}C NMR spectrum that corresponded to the symmetrical structure of the diol.

According to DSC data, **PEAs 3** were amorphous and showed a rather high T_g (up to 102°C, Table 2),. when compared with the analogous samples obtained from α,ω-alkylene diols (max. T_g = 59°C) [2]. According to GPC data many samples of **PEAs 3** have rather high molecular masses and narrow polydispersities (Table 2). They were soluble in common organic solvents like chloroform and tetrahydrofuran. Though films were cast from chloroform solutions

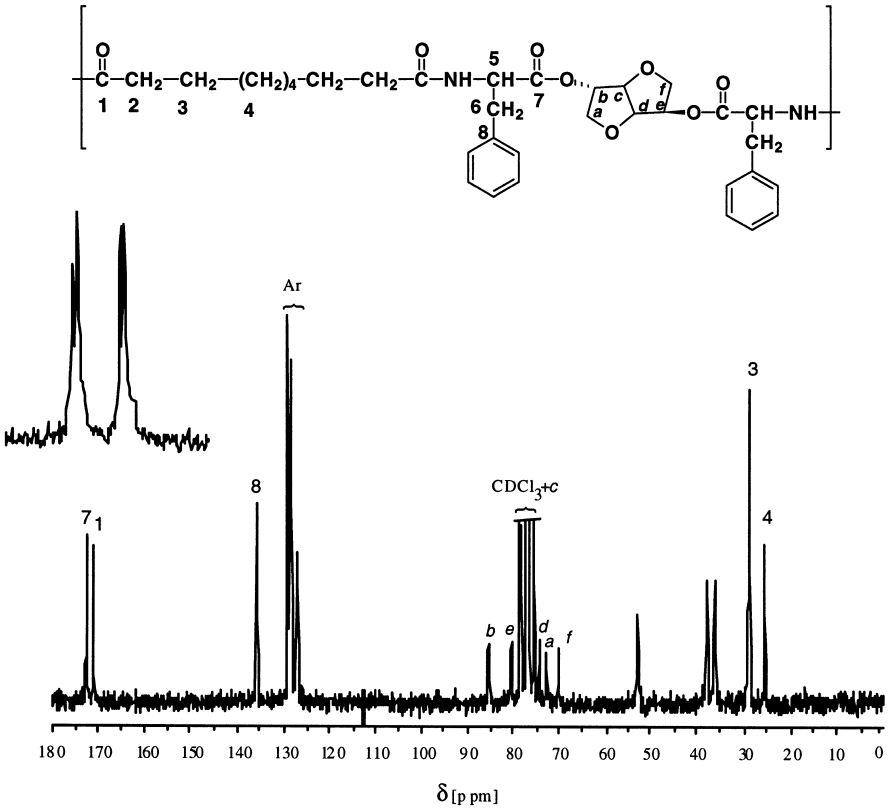


Figure 1. 25,4 MHz ^{13}C-NMR spectrum of **3-DAS, Phe (8).** (No. 3, Table 1).

of all the samples having M_w higher than 15.000 (i.e., with the exception of samples numbers 5 and 10, Table 1).

Hydrolysis Studies

The enzyme catalyzed *in vitro* hydrolysis of the **PEAs 3** obtained was carried out using potentiometric titration of carboxyl groups resulting from the cleavage of ester groups as described previously [1-3]. Films with d = 5 cm and ~ 200 mg were used for this study.

Two types of hydrolases, α-chymotrypsin and lipase, were used as enzymes. The hydrolysis was studied under the conditions close to the physiological ones – 0.1 N NaCl solution, pH = 7.4, and t = 37°C. The tendency of the **PEA** sample to rapid hydrolysis was characterized by the consumption of NaOH within 70 minutes.

TABLE 3. Enzyme Catalyzed *in vitro* Hydrolysis of **PEAs 3**[a)]

#	PEAs 3	Enzyme	NaOH consumption μmol /70 min		$Ve \cdot 10^2$ [b)] mg /cm^2.h
			Enzyme in solution 2mg/5ml	Surface immobilized Enzyme	
1	**3-DAS,Phe(4)**	α–Chymotrypsin	53 ± 6	16 ± 2	32
2	**3-DAS,Phe(4)**	Lipase	35 ± 8	11 ± 1.5	21
3	**3-DAS,Phe(6)**	α–Chymotrypsin	16 ± 3	11 ± 2	10
4	**3-DAS,Phe(8)**	α–Chymotrypsin	8 ± 1.5	7 ± 0.5	5.3
5	**3-DAS,Phe(10)**	α–Chymotrypsin	2 ± 1	0	
6	**3-DAS,Leu(4)**	α–Chymotrypsin	6 ± 1	3 ± 0.3	3.2
7	**3-DAS,Leu(8)**	α–Chymotrypsin	5 ± 0.5	2 ± 0.2	2.9
8	**3-DAM,Phe(4)**	α–Chymotrypsin	54 ± 5	21 ± 2	32
9	**3-DAM,Phe(4)**	Lipase	52 ± 6	17 ± 1	31.3
10	**3-DAM,Phe(8)**	α–Chymotrypsin	17 ± 3	10 ± 0.8	11

a) Films cast from chloroform solution. d = 5 cm, m ~ 200 mg. Potentiometric titration, 0.1 N NaCl, pH = 7.4, t = 37°C.

b) V_e – average erosion rate for the "enzyme in solution". For the films with d = 5 cm $\mathbf{V_e = 1.092 \cdot 10^{-5} \cdot M \cdot a}$, where **M** is molecular weight of the elemental link of **PEA 3**, **a** - **NaOH** consumption in μmol **/70** min.

Table 3 summarizes the results of the α-chymotrypsin and lipase catalyzed hydrolysis of the newly synthesized **PEAs** for an interval of 70 minutes for both the dissolved and surface-immobilized enzymes.

Enzymes in Solution

In a previous publication [2], we have found that among **PEAs** based on hydrophobic α-amino acids those **PEAs** based on L-phenylalanine showed the highest tendency towards the α-chymotrypsin catalyzed hydrolysis followed by L-leucine derivatives. The highest hydrophobicity of the benzyl side groups in the phenylalanine-based **PEAs** was suggested to be responsible for this finding. The same is true for **PEAs 3**. The polymers based on phenylalanine revealed the highest tendency towards the α-chymotrypsin catalyzed hydrolysis, and this tendency is very close for **DAS** and **DAM** derivatives. It should be noted that the tendency to the α-chymotrypsin catalyzed hydrolysis of **PEAs 3** based on L-phenylalanine is higher, and based on L-leucine is lower than the tendency of analogous **PEAs** composed of α,ω-alkylene diols [2]. Lipase was found to be as effective as α-chymotrypsin in the hydrolysis of **PEAs 3**. Thus, one can conclude that the rigid bicyclic fragments of **DAHs** don't prevent the enzyme catalyzed hydrolysis of **PEAs 3**.

Immobilized Enzymes

Active enzymes were spontaneously absorbed (immobilized) onto the surface of polymeric films (with the exception of **1-DAS,Phe(10)** where no active enzyme adsorption was observed). These immobilized enzymes catalyzed the hydrolysis with a rather high rate ranging from 30% (sample 1) to 92% (sample 4) of the "*enzymes in solution"*. Lipase was immobilized onto the film surface as well. The hydrolysis rates of **PEAs 3** by immobilized enzymes is at the same level as the hydrolysis rates of **PEAs** composed of α,ω-alkylene diols [2].

The enzyme catalyzed average erosion rates V_e of **PEAs 3** in both cases ("*enzymes in solution" and* "*immobilized enzymes"*) were very close to the erosion rates of polyanhydrides [11].

CONCLUSION

It may be noted that the tendency of the **PEAs 3** to undergo α-chymotrypsin catalyzed hydrolysis decreases with the larger methylene chain of the diacid's, i. e., with increasing hydrophobicity of the diacid residue in the polymer backbone. This property contrasts with this result can be explained by competitive interaction of hydrophobic acyl residue with the hydrophobic sites of the enzyme leading to non-productive binding and decreasing overall hydrolysis rate [12]. The retardation in case of **1-DAS,Phe(10)** was extraordinarily high, and virtually no hydrolysis was observed under the experimental conditions used (the NaOH consumption 2 μmol/70 minutes is within the experimental error).

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