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Synthesis and characterization of a polytartaramide based on L-lysine

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The synthesis of a polyamide entirely based on naturally occurring compounds has been carried out by polycondensation of *N,N*-bis(trimethylsilyl) L-lysine ethyl ester and bis(pentachlorophenyl) di-*O*-methyl-L-tartrate in a chloroform solution. The new polytartaramide has a molecular weight of ~6000, displays high optical activity and is soluble in water. The regioregularity of the polymer has been examined by ^{13}C n.m.r. with the support of model compounds, which revealed that the chain is predominantly syndioregic in nature.

(Keywords: chiral polyamides; optically active polyamides; L-lysine)

INTRODUCTION

The interest in polyamides based on naturally occurring products is steadily growing due to their potential as non-toxic biodegradable materials in the biomedical field. Amongst the different natural sources that are easily available, carbohydrates and α-amino acids stand out as the compounds most frequently used as precursors. Aldaric acids and dibasic α-amino acids appear particularly convenient for the synthesis of polyamides of AABB type as evidenced by the number of polyaldaramides[1–4] and lysine-based polyamides[5–8] that have been described so far. The synthesis of such polyamides implies the preparation of suitably protected monomers and usually precludes the use of direct methods of polycondensation requiring aggressive conditions. Therefore, unconventional techniques capable of rendering high molecular weight products under mild conditions should be applied.

Recently we have reported on optically active polytartaramides, i.e. polyamides derived from tartaric acid, that were obtained by polycondensation of bis(pentachlorophenyl)-L-tartrates with 1,n-alkanediamines activated as *N,N'*-bis(trimethylsilyl) derivatives[9,10]. It was found that activation of both comonomers turns out to be a convenient strategy for achieving stereoregular polymers with satisfactory molecular weights. Some of these polytartaramides may undergo hydrolysis under mild conditions and exhibit physical properties comparable to those of commercial nylons[11]. However, the potential development of these compounds as degradable biomaterials will be partly limited, since aliphatic diamines are still relatively toxic. An interesting approach to the obtention of fully biocompatible polytartaramides would be the utilization of diamines of natural origin, as in the case of L-lysine. Moreover, the presence of lysine residues in the polyamide offers the additional interest of allowing bioactive ligands to be reversibly attached to the polymer via the carboxylate side groups.

Interest in L-lysine as a building block to obtain polyamides is by no means new. L-Lysine has been condensed with adipic acid by interfacial methods to produce optically active polyamides with regular structural sequences[5]. Unfortunately polyamides produced by such a procedure possess viscosity characteristics that are too low for them to be promising from a practical point of view. More recently, Katsarava *et al.*[7] have described the synthesis of a series of AABB aliphatic polyamides containing L-lysine by activating the two comonomers taking part in the polymerization. Using this method, polymers with intrinsic viscosities $[\eta]$ in the range 0.25–0.70 dl g^{-1} were attained in high yields. For example, the polycondensation of N^{α},N^{ε}-bis(trimethylsilyl)lysine ethyl ester with bis (*p*-nitrophenyl) succinate afforded a polyamide with $\eta = 0.5$ dl g^{-1} in 71% yield.

In this paper we report the synthesis of a new polytartaramide based entirely on naturally occurring products. L-Tartaric acid and L-lysine have been conveniently derivatized and subjected to polycondensation in a solution to give the highly substituted polyamide poly[(*S*)-1(5)-ethoxycarbonyl-pentamethylene-di-*O*-methyl-L-tartaramide], abbreviated PLYTA, with a molecular weight sufficient for the purpose of this work. This polyamide has been fully characterized and a close examination of the microstructure with regard to chain regioregularity has been carried out by means of ^{13}C n.m.r. spectroscopy. Suitable model compounds have been prepared in order to assist the interpretation of n.m.r. data.

EXPERIMENTAL

All reagents were analytical grade or higher and were used without further purification. Solvents to be used under anhydrous conditions were exhaustively dried by standard methods. Viscosities were measured in dichloro-

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acetic acid at 25.0±0.1°C by using an Ubbelohde microviscosimeter. Combustion analyses were carried out by the CID, Barcelona. Molecular weight distributions were determined by g.p.c. in a Waters Associates instrument fitted with a 10^3 and 10^2 nm PL gel column set and using chloroform as eluent; calibration was made against polystyrene standards. I.r. spectra were regarded on a Perkin-Elmer 2000 spectrometer from samples in KBr discs or films cast in chloroform. N.m.r. spectra were recorded on a Bruker AMX-300 spectrometer operating at 300.13 and 70.48 MHz for ^1H and ^{13}C, respectively. Tetramethylsilane was used as internal reference standard and the internal lock was $CDCl_3$. Thermal experiments were carried out at heating rates of 20°C min^{-1} under a nitrogen atmosphere, on a Perkin–Elmer DSC-4 instrument calibrated with indium. Optical rotations were recorded on a Perkin–Elmer 141 polarimeter at the sodium D line (589 nm) at 23°C. Circular dichroism spectra were obtained in trifluoroethanol (TFE) at 25°C, on a Jasco J-700 apparatus fitted with a cell of an optical path length of 1 mm.

Synthesis of monomers. Commercial L-lysine monohydrochloride was esterified by treatment with thionyl chloride and ethanol. *N,N'*-Bis(trimethylsilyl)-L-lysine ethyl ester (I) was synthesized according to the standard method used by us for the trimethylsilylation of diamines which has been described in detail elsewhere[9,10]. In brief, a mixture of L-lysine ethyl ester dihydrochloride (0.02 mol), trimethylsilyl chloride (0.04 mol) and triethylamine (0.16 mol) in dried toluene (50 ml) was refluxed for 5 h under a nitrogen atmosphere. The reaction mixture was then filtered and the filtrate evaporated to dryness. Distillation of the liquid residue under diminished pressure gave I (120–130°C, 0.1 mm Hg) as a colourless oil in 63% yield.

As reported elsewhere[9,10], bis(pentachlorophenyl) di-*O*-methyl-L-tartrate (II) was obtained from commercial diethyl L-tartrate by a synthetic route consisting of three steps. In brief, the diethyl ester was methylated with dimethyl sulfate, then hydrolysed to di-*O*-methyl-L-tartaric acid, and finally converted into II by treatment with thionyl chloride and pentachlorophenol. The overall yield of this synthesis is about ~45%.

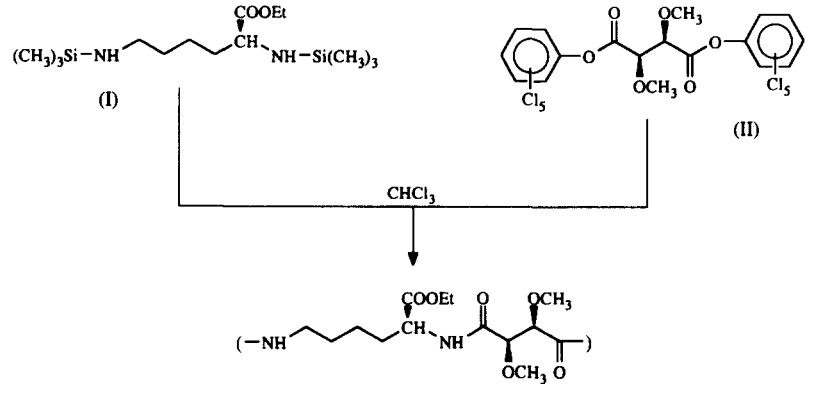
Synthesis of PLYTA. To a stirred solution of I (4 mmol) in dried chloroform (8 ml) cooled at 0°C, 4 mmol of compound II were added in small portions and the solution allowed to reach room temperature. After stirring for 3 days under rigorous exclusion of moisture, the reaction mixture was refluxed for 1 h and then poured into ethyl ether (100 ml). The precipitated polymer was recovered by centrifugation as a yellowish powder which was then repeatedly washed with ethyl ether and finally dried under vacuum (yield ~100%). Analysis calculated for $(C_{14}H_{24}N_2O_6.1/2H_2O)_n$ [$(325.17)_n$]: C,51.67; H,7.75; N,8.61. Found: C,51.43; H,7.37; N,8.41. $[\alpha]_D^{23}$: +71.9° (c = 0.487, in chloroform). I.r. (cm^{-1}, film from TFE): 3292 (amide A), 3070 (amide B), 1743 (C=O, ester), 1661 (amide I), 1526 (amide II), 1190 (C–O, ester), 1096 (C–O, ether). ^1H n.m.r. (300.13 MHz): δ 7.22 (m, N*H*CH, 1H), 6.84 (m, br, CH_2NHCO, 1H), 4.55 (m, CONHC*H*, 1H), 4.21 (m, br, NHCOC*H*, and $COOCH_2$, 3H), 3.45 (m, OCH_3, 6H), 3.26 (m, H^{ε}, 2H), 1.93–1.77 (dm, H^{β}, 2H), 1.57 (m, H^{γ}, 2H), 1.46 (m, H^{δ}, 2H), 1.28 (t, CH_2CH_3, 3H). ^{13}C n.m.r. (70.48 MHz): δ 171.6 (COO), 169.5–168.8 (CONH), 82.3–81.4 (*C*HCONH), 60.4 ($COOCH_2$), 59.4–59.2 (OCH_3), 51.8 (CONHCH), 38.0 (C^{ε}), 30.1 (C^{β}), 28.5 (C^{δ}), 22.5 (C^{γ}), 13.9 (CH_2CH_3).

Preparation of model compounds. Commercial L-norleucine and 6-aminocaproic acid were esterified by treatment with thionyl chloride and ethanol. By reaction of the resulting ethyl esters with bis(pentachlorophenyl) 2,3-di-*O*-methyl-L-tartrate and triethylamine in chloroform at room temperature for 24 and 12 h, respectively, compounds *N,N'*-bis[(S)-1-ethoxycarbonyl-pentyl]-2,3-di-*O*-methyl-L-tartaramide ($\alpha\alpha$ model) and *N,N'*-bis(6-ethoxycarbonyl-pentyl)-2,3-di-*O*-methyl-L-tartaramide ($\varepsilon\varepsilon$ model) were obtained. In a similar manner, the mixed tartaramide ($\alpha\varepsilon$ model) was prepared by reaction of the active diester (1 mol) with the norleucinate (0.75 mol) followed by reaction with the aminocaproate (1.25 mol). The resulting reaction mixture contained the three model compounds $\alpha\alpha$, $\varepsilon\varepsilon$ and $\alpha\varepsilon$ in an approximate ratio of 1:2:2, as visually estimated by t.l.c. (R_f: 0.65, 0.10 and 0.32, in ethyl acetate:hexane 1:1). After purification, the mixture without resolving was used for spectroscopic analysis.

RESULTS AND DISCUSSION

Synthesis and characterization

Polycondensation of ethyl L-lysinate activated as *N,N'*-bis(trimethylsilyl) derivative (I) with the bis(pentachlorophenyl) diester of L-tartaric acid (II) afforded PLYTA, as shown in *Scheme 1*. Polycondensation results



PLYTA

Scheme 1

obtained in a variety of solvents are compared in *Table 1*. The best results were achieved in chloroform, very likely due to the fact that both comonomers as well as the forming polymer were soluble in this solvent. A limiting viscosity number of 0.39 was measured for PLYTA obtained under such conditions, which corresponds to a molecular weight of ~5000 if the viscosimetric parameters of polyamide 6,6[12] are used for calculation. On the other hand, a weight-average molecular weight of 6000 with a polydispersity of 1.9 was determined for this polyamide by g.p.c. calibrated against polystyrene standards. Such figures compare well with those given for other L-lysine containing polyamides obtained by the same methodology[7]. However, such molecular weights turn out to be markedly lower than those achieved in the synthesis of polytartaramides made of aliphatic 1,n-alkanediamines, for which intrinsic viscosities up to 2.3 dl g^{-1} were found[10]. These results infer that the reactivity of the amino groups of L-lysine towards condensation is diminished when compared with aliphatic diamines. This is to be expected if the deactivating effect of the electron-withdrawing ethoxycarbonyl group in lysine is taken in account.

Table 1 Results of polycondensations of I with II in different solvents

Solvent	Reaction medium phase[a]	Yield (%)	η_{sp}/c (dl g^{-1})[b]	M
DMF	heter./hom.	96	0.34	–
CH_3CN	heter./heter.	90	–	4700[d]
HMPT	heter./heter.	–	0.38	–
$CHCl_3$	hom/hom.	>99	0.45	5000[c], 6000[d]

[a] Refers to the phase of the reaction medium at the beginning and at the end of the polycondensation
[b] Measured in dichloroacetic acid at $c = 0.5$ g dl^{-1} and $T = 25°C$
[c] Calculated by applying the equation $100[\eta] = 0.5 + 0.352M^{0.551}$
[d] Measured by g.p.c.
DMF, *N-N*-dimethylformamide
HMPT, hexamethylphosphoramide

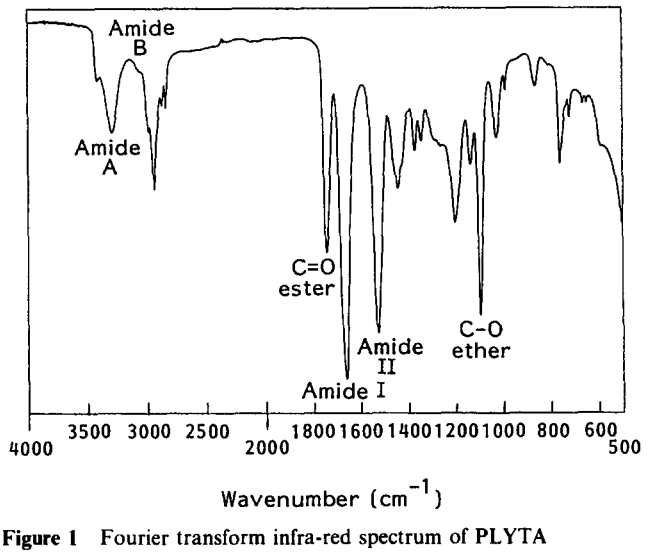


Figure 1 Fourier transform infra-red spectrum of PLYTA

The polymer is soluble in a wide variety of solvents including those usually known for polyamides [formic acid, dimethylformamide (DMF) and dimethylsulfoxide (DMSO)] as well as in chloroform and in ethanol. It is also soluble in hot water. Elemental microanalyses of PLYTA obtained in chloroform are in full agreement with the chemical constitution expected for this polyamide provided that 0.5 mol of water is added per molecular formula of the repeating unit of the polymer. The presence of moisture, unable to be removed by standard drying methods, appears to be usual in polytartaramides. In fact, similar amounts of bound water were reported to be present in poly(alkylene-di-*O*-methyl-L-tartaramide)s for alkylene chains comprised of less than six methylene units[10].

The i.r. spectrum of PLYTA (*Figure 1*) contains the characteristic bands of polyamides together with those corresponding to ethoxycarbonyl and methoxyl side groups attached to the lysine and tartaric acid moieties, respectively. The absorption at 3420 cm^{-1} observed as a shoulder of the amide A band is also found in other polytartaramides[10], which is currently interpreted as arising from NH hydrogen-bonded to methoxyl side groups. No traces of absorptions indicative of chain end groups, in particular of pentachlorophenyl ester, are detected in the spectrum.

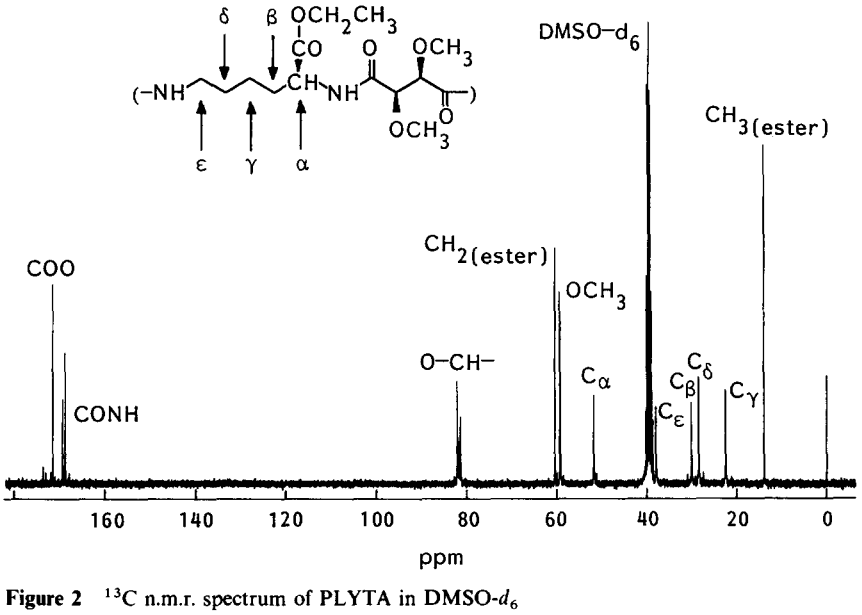


Figure 2 ^{13}C n.m.r. spectrum of PLYTA in DMSO-d_6

The presence of three asymmetric centres per repeating unit of PLYTA together with the occurrence of regioisomerism associated with the orientation of the lysine residue along the chain, accounts for the complexity observed in ^{1}H n.m.r. spectra of this polymer. Nevertheless, a satisfactory interpretation of proton spectra could be made on the basis of the signal assignments known for L-lysine[14] and with the help of ^{1}H-^{1}H homonuclear correlation experiments. As indicated in the Experimental section, every signal could be assigned in conformity with the chemical constitution expected for this polyamide.

The ^{13}C n.m.r. spectrum of PLYTA (*Figure 2*) contains 11 well separated signals arising from the corresponding 11 chemically different carbons contained in the constitutional repeating unit of the polymer. However, a close inspection of the spectrum reveals multiplicities for certain signals attributable to the occurrence of regioisomerism. We will make use of these signals for evaluating the regiochemical disorder of PLYTA; this aspect will be discussed in detail in the next section.

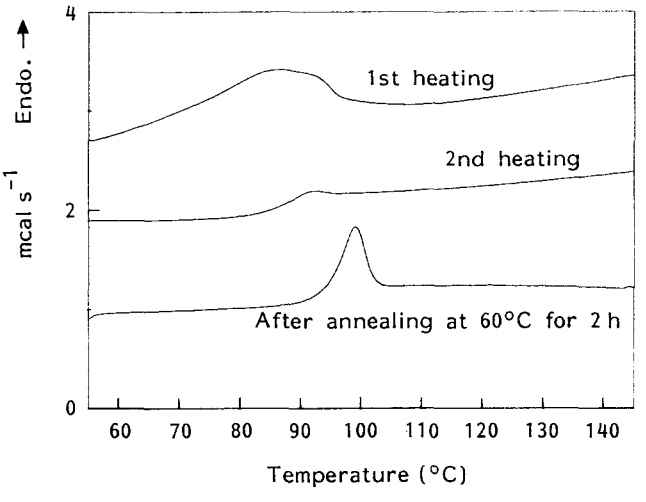


Figure 3 D.s.c. traces of PLYTA under the indicated conditions

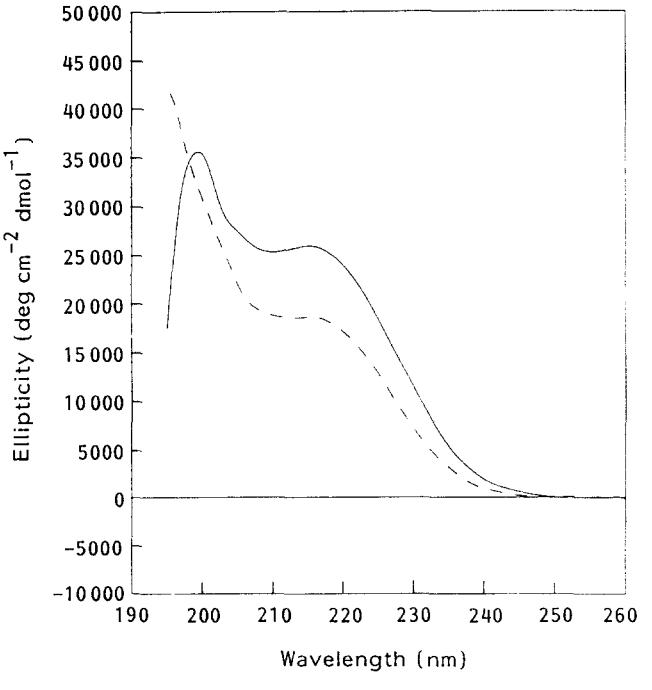


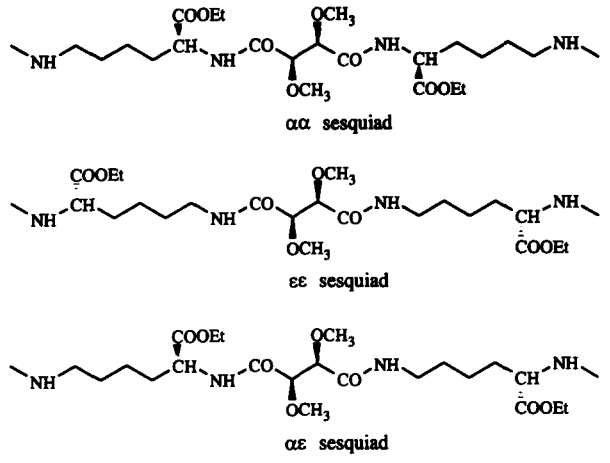
Figure 4 Circular dichroism spectrum of PLYTA in TFE (—) compared with the spectrum obtained for poly(pentamethylene-di-2,3-*O*-methyl-L-tartaramide) in the same solvent[10] (– – –)

The d.s.c. trace of PLYTA (*Figure 3*) recorded during heating at 20°C min^{-1} contains an endotherm centred at 85°C and reveals that decomposition of the polymer begins near to 210°C. Melting points in the range 83–113°C have been reported for polyamides prepared by condensation of lysine with adipic acid containing both regular and non-regular sequences[5]; they were visually determined as corresponding to the temperature at which the polymer became semi-molten. Since no indication of crystallinity in PLYTA was detected either by X-ray diffraction or polarized optical microscopy, no melting transition should be expected for this polymer and a different phenomenon must be at the origin of the endothermic peak observed by d.s.c. Second heating thermograms recorded after rapid cooling of the sample to room temperature do not reproduce the endotherm observed during the first heating cycle and show only a well-defined slope change at 86°C, clearly corresponding to the glass transition of the polymer. When a sample is subjected to annealing at a temperature of 60°C for a few hours, the reheated trace displayed the endotherm peak slightly displaced to higher temperatures and noticeably increased in size. These observations clearly indicate that a structural relaxation process, common amongst linear amorphous polymers and widely known as physical ageing[15], must be responsible for the heat absorption taking place in the proximity of the glass transition temperature.

A specific optical rotation of +71.9°C was measured for a solution of PLYTA in chloroform. This value turns out to be much higher than those reported for the L-lysine-containing polyalkanamides[7] but comparable to those displayed by other polytartaramides based on aliphatic diamines[10]. The value is also significantly higher than those displayed by either of the two comonomers I (−8.4°) and II (−38.25°) employed for building the polymer. On the other hand, a circular dichroism spectrum containing a maximum at 215 nm was registered from a solution in TFE (*Figure 4*). The shape of this spectrum closely resembles those reported for poly(alkylene-di-*O*-methyl-L-tartaramide)s, a family of polymers that is believed to adopt regular conformations in such a solvent[10].

Regioisomerism in PLYTA

Although both comonomers used for construction of PLYTA are optically active, they behave in a completely different manner with regards to the stereochemistry of the forming polymer chain. Since L-tartaric acid is dissymetric, i.e. contains a two-fold axis normal to the backbone of the molecule, a unique arrangement is possible for this residue in the polymer chain, which will therefore have a threodiisotactic configuration. In contrast, two orientations are allowed for the L-lysine residue depending on the way in which the monomer is added onto the growing chain. As a consequence, three different structures are feasible for a sesquiad consisting of one diacid and two diamine residues, which may be denoted $\alpha\alpha$, $\varepsilon\varepsilon$ and $\alpha\varepsilon$ in reference to which of the two NH of lysine is implied in each amide group (*Scheme 2*). Whereas pure syndioregic chains will consist of an alternating sequence of $\alpha\alpha$ and $\varepsilon\varepsilon$ structures, only $\alpha\varepsilon$ sesquiads should



Scheme 2

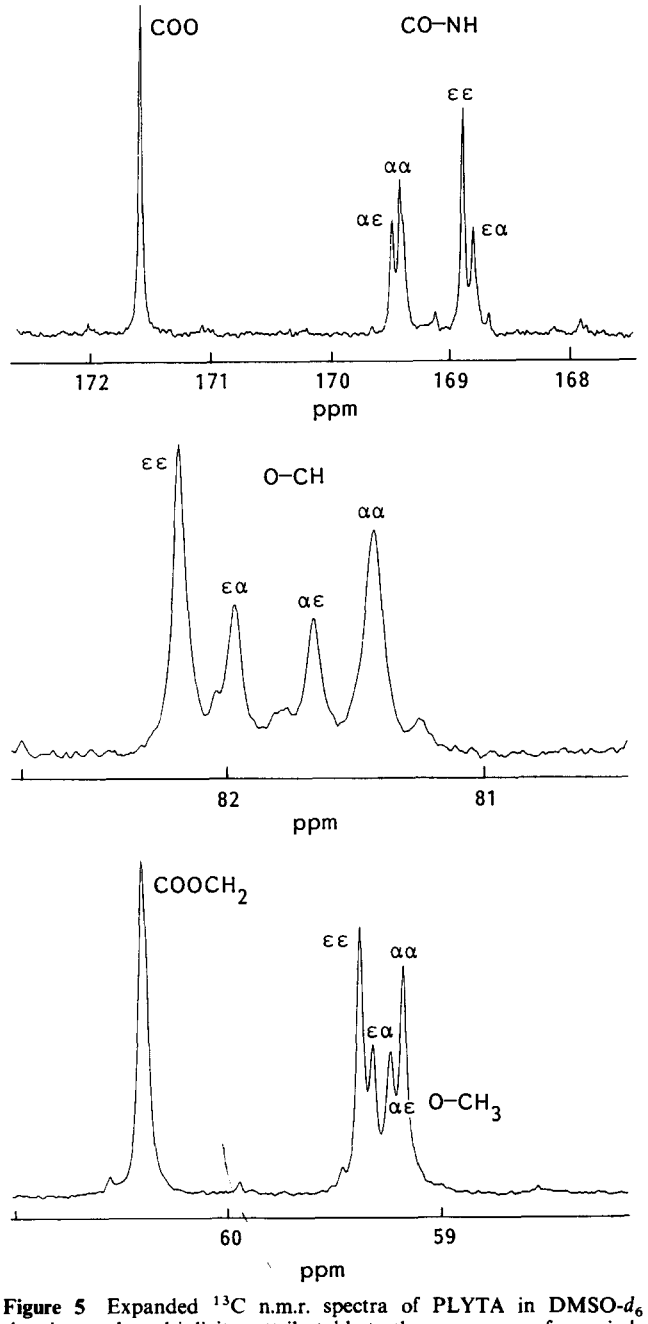
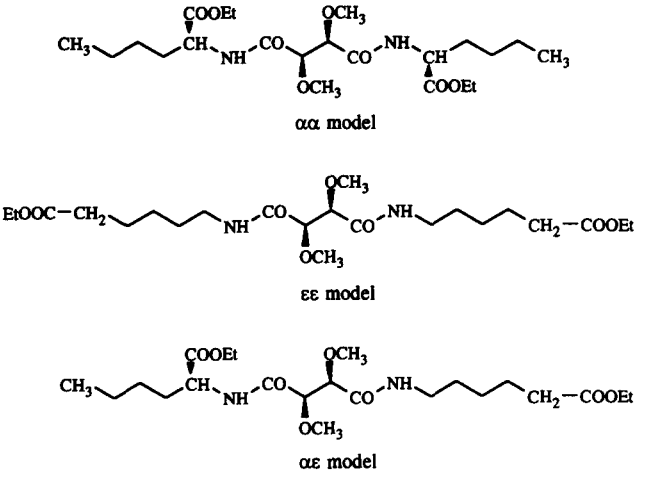


Figure 5 Expanded ^{13}C n.m.r. spectra of PLYTA in DMSO-d_6 showing peak multiplicites attributable to the occurrence of sesquiads as defined in *Scheme 2*

be present in an isoregic polymer. On a statistical basis, the fraction contents in the three possible structures should be 1:1:2 for a perfectly aregic polymer chain. However, a certain deviation from the random statistical distribution may be reasonably anticipated for the orientation of lysine in PLYTA due to the difference in reactivity between α and ε amino groups.

^{13}C n.m.r. spectra of PLYTA exhibited splitting of peaks for reasonances of the three different carbons (CH, CH_3 and CO) contained in the diacid moiety. Four different chemical environments, one for each symmetric



Scheme 3

Table 2 ^{13}C n.m.r. assignments for carbon atoms in the diacid unit of PLYTA and model compounds

Signal	Chemical shift (ppm)		Assignment
	Model compounds	PLYTA	
NH–CO	169.33	169.48	αε
	169.15	169.41	αα
	168.81	168.88	εε
	168.84	168.80	εα
O–CH	82.22	82.18	εε
	82.08	81.97	εα
	81.71	81.66	αε
	81.51	81.42	αα
$O–CH_3$	59.34	59.38	εε
	59.27	59.32	εα
	59.08	59.08	αε
	58.95	59.17	αα

Table 3 ^{13}C n.m.r. signal intensities for carbon atoms in the diacid unit of PLYTA

Signal	Chemical shift (ppm)	Fraction of sequences			
		αα	αε	εα	εε
NH–CO	168–169	0.320	0.170	0.200	0.320
O–CH	81–82	0.320	0.200	0.160	0.320
OCH_3	58–59	0.310	0.180	0.190	0.310
Average		0.317	0.183	0.183	0.317

structure ($\alpha\alpha$ and $\varepsilon\varepsilon$) and two for the asymmetric $\alpha\varepsilon$ structure, are possible for each of such carbons provided that shielding effects arising from neighbouring sesquiads are negligible. The expanded ^{13}C n.m.r. spectra of methyl, methyne and carbonyl carbons contained in the diacid unit are shown in *Figure 5*, where labels denote both the type and relative position of amino groups attached to the unit in each case. Peak assignments can be carried out without ambiguity by comparison to the spectra of model compounds represented in *Scheme 3*. It is worth noting that relative chemical shifts corresponding to the four distinguishable environments depend upon the carbon atom concerned; whilst the same sequence of shielding effects is observed for methyne and methyl carbons, an inverted order is found for the carbonyl carbons (*Table 2*). By a comparative estimation of peak intensities (*Table 3*), an $\alpha\alpha$ or $\varepsilon\varepsilon$ structure content 1.7 times higher than that of the $\alpha\varepsilon$ structures is inferred, which indicates that PLYTA is predominantly syndioregic. On the other hand, splitting of the signal at 51.9 ppm, ascribed to the asymmetric carbon of lysine is also detected. The relative intensities of the two subpeaks contained in this signal confirms the predominant presence of $\alpha\alpha$ and $\varepsilon\varepsilon$ sesquiads. In view of these results, it may be concluded that the higher nucleophilicity of the ε-NH group of lysine should favour the formation of α-NH-ended oligomers during the earlier stages of polycondensation. This will lead to a departure from the Bernouillian statistical distribution and could account for the relatively low molecular weight achieved for these polytartaramides, when compared to those prepared from symmetric diamines.

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