Polymer Bulletin 14, 187–192 (1985)

Polymer Bulletin

Drug Carriers

Optically Active Poly (β-Malic-Acid)

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Summary

A simple and reproducible method of synthesizing enantiomers of benzyl malolactonate is described starting from optically active aspartic acid. Chiral benzyl malolactonate is a β-substituted β-lactone monomer which can be readily polymerized anionically using triethylamine as the initiator to yield poly(benzyl β-malate) which is an optically active, semicrystalline polymer. Cleavage of protecting benzyl ester groups yields optically active poly(β-malic acid). The properties of the racemic and optically active monomers and polymers are compared. Optically active (-)poly(β-malic acid) shows one accessible positive CD band in the far UV.

Introduction

Increasing interest is being devoted to the use of polymeric drug carriers in drug delivery systems for time-controlling drug delivery, for directing drugs or other therapeutic molecules to specific sites, and for protecting the biological environment from toxic drugs. Many synthetic polymers have been evaluated in drug-carrier systems including polymers with polyacrylic or polymethacrylic-type backbones which have received much attention because of the presence of carboxylic acid pendent groups for drug attachment and for solubilization in water (1). However, these polymers are biostable, a property which is regarded more and more as a shortcoming. A few years ago, poly(β-malic acid) (PMLA 100) was selected as a suitable candidate for drug-transportation and design of macromolecular prodrugs because of the potential biodegradability of the poly(β-hydroxy acid)-type backbone with pendent acidic groups (2).

PMLA 100 was synthesized for the first time in 1978 (3) starting from bromosuccinic acid which was converted to benzyl malolactonate, a β-substituted β-lactone, and the latter was polymerized by a ring opening polymerization. However, only the racemic form was prepared either because racemic bromosuccinic acid was used as the starting compound or because racemization occured during the synthesis of the β-lactone monomer from optically active bromosuccinic acid.

Recently, an attempt was made to prepare optically active poly(β-malic acid) directly from L-malic acid (4). However, only very low molecular weight oligomers were obtained from optically active benzyl malolactonate, MLA*BE,

of low optical purity and relatively low bulk purity. Efforts to improve on the method of monomer synthesis remained unsuccessful (5). In this report, we describe a new and reproducible method to synthesize optically active benzyl malolactonate with high optical purity and high bulk purity starting from L-aspartic acid. It is also shown that the polymerization of resulting MLA*BE yields isotactic optically active poly(benzyl β-malate), PMLA*BE, and that selective catalytic cleavage of benzyl ester groups is feasible and provides optically active poly (β-malic acid), PMLA*100.

Experimental

To an ice-cold mixture of L-aspartic acid (1, 100 g) and NaBr (416 g) in 2N H_2SO_4 (500 ml), $NaNO_2$ (62 g) was added progressively over a period of 1.5 hours. The whole mixture was further stirred for 30 mn. at room temperature. After addition of urea (10 g) to decompose the excess reagent, bromosuccinic acid was extracted with ethyl acetate. The combined extracts were washed with slightly acidic water and dried over anhydrous sodium sulfate. Crude L-bromosuccinic acid (2) was recovered (yield = 78 %, Mp = 169°C, $[\alpha]_D^{25}$ = -69 deg.cm^2.dg^{-1} (c = 6 g.100 cm^{-3} in ethyl acetate), $[\alpha]_D^{25}$ = -40 (c = 1.4 g.100 cm^{-3} in water).

L-(-)-bromosuccinic acid (85 g) was dissolved in anhydrous tetrahydrofuran (150 ml) containing trifluoroacetic anhydride (100 g) at 10°C. The mixture was allowed to stirr for 1.5 h. Tetrahydrofuran and trifluoroacetic acid were then distillated under vacuum to yield L-(+)-bromosuccinic anhydride (3), a white crystalline compound (mp = 68°, $[\alpha]_D^{25}$ = + 5.9 (c = 1 g.100 cm^{-3} in THF)). Benzyl alcohol (54 g) was added to (+)-bromosuccinic anhydride and the mixture was allowed to react for 4 h. at room temperature and 1 h at 60°C. After completion, the reaction yielded a mixture of optically active bromosuccinic acid monobenzyl ester isomers (4 and 5). The resulting mixture was dissolved in water (500 ml) and sodium hydroxide was added to rise pH up to 7.8. Benzene (300 ml) was added and the biphasic system was allowed to stirr overnight at 50°C. After cooling, the organic layer was separated from the aqueous phase and crude optically active benzyl malolactonate was recovered after evaporation of the solvent. The resulting liquid was purified using preparative HPLC (SiO_2 μ-Bondapack, dichloromethane) and further vacuum distilled twice (bp=90°C; 10^{-3}mm Hg). 6g of liquid benzyl malolactonate (6) was recovered ($[\alpha]_D^{25}$ = +4.3 (c = 2 g.100 cm^{-3} in dioxane)). Infrared and ^1H NMR spectra of MLA*BE feature characteristics similar to those of racemic MLABE (C=O (ester + lactone) at 1750 cm^{-1}, C=O (lactone) at 1820 cm^{-1} ; ^1H NMR : 3.6 (m), ; 4.8 (t), 5.25 (s), 7.35 (s) ppm in $CDCl_3$).

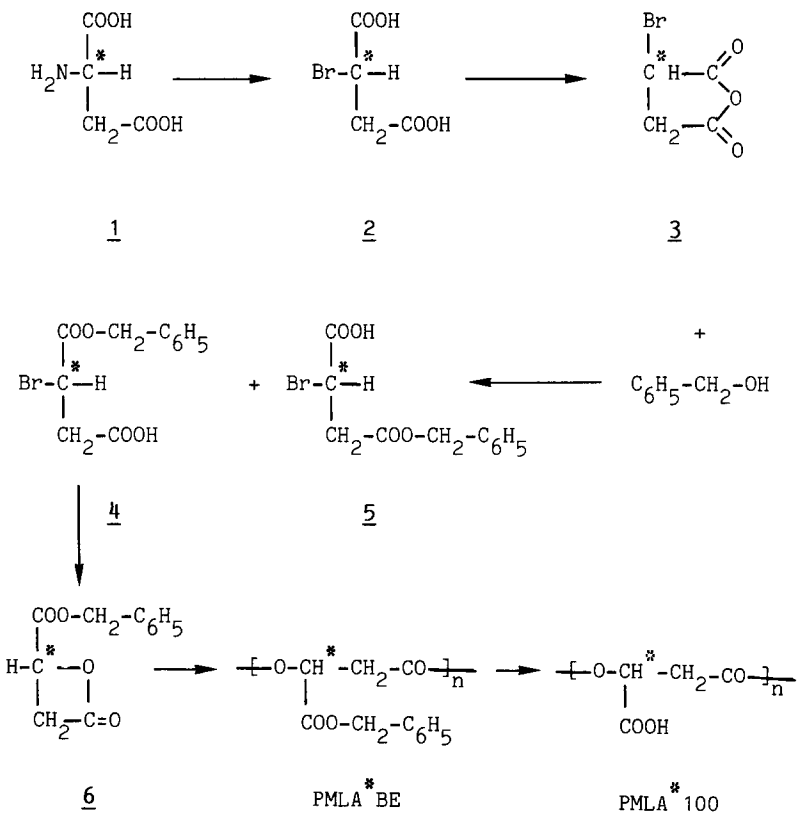
Optically active benzyl malolactonate was polymerized at 70°C for 24 h under nitrogen atmosphere, using triethylamine as the initiator. The resulting poly(benzyl β-malate), PMLA*BE, ($M_{GPC} \simeq$ 6,000 as measured in dioxane by GPC on μ-Styragel in regard to polystyrene standards) was optically active ($[\alpha]_D^{25}$ = -5.0 (c = 1 g.100 cm^{-3} in CH_2Cl_2)). Conversion to optically active poly(β-malic acid), PMLA*100, was carried out by catalytic hydrogenolysis in N-methylpyrrolidone at room temperature using a 10 % Palladium-charcoal catalyst. After conversion to sodium salt by addition of suitable amounts of NaOH 1M, dialysis and freeze-drying, 1.5 g solid optically active PMLA*100 Na was recovered and stored in a dessicator ($[\alpha]_D^{25}$ = +4.5 (c = 0.88 g.100cm^{-3} in water).

ORD spectra were recorded with a FICA "Spectropol I" spectropolarimeter thermostated at 25°C. CD spectra were recorded using a JASCO J40B dichrometer at room temperature. NMR spectra were recorded using a BRUKER WM 250 FT.

Discussion

The synthesis of racemic benzyl malolactonate is based on the ring closure of either silver or better of sodium salts of α-monobenzyl bromosuccinate (3-6). The first attempts to prepare optically active benzyl malolactonate from optically active bromosuccinic acid by using the acetyl chloride method failed because of racemization at the stage of conversion of the optically active bromosuccinic acid to the corresponding anhydride (7).

We have recently found that the reaction of optically active bromosuccinic acid with trifluoroacetic anhydride yields optically active bromosuccinic anhydride at room temperature. This finding provides a route to the synthesis of optically active malic acid polymers from natural, readily available L-aspartic acid according to the following series of reactions :



The first step, the substitution of the amino group with the bromine atom, occurs with inversion of configuration. The product obtained, 2 , remains of high optical purity (8). 2 is then converted to bromosuccinic anhydride at rather low temperature (10°C) using trifluoroacetic anhydride. The following steps are identical to those used to synthesize the racemic monomer: monoesterification with benzyl alcohol followed by lactonization of the α-bromosuccinic acid monobenzyl ester isomer. The optically active benzyl malolactonate prepared from L-aspartic acid is a dextrorotatory liquid at room temperature whose enantiomeric excess (L-D/L+D) was 80 % as evaluated by 250 MHz ^{1}H-NMR in the presence of tris[3-heptafluoropropyl hydroxy methylene)-d-camphorato], Europium (III). The β-substituted β-lactone was polymerized in bulk with triethylamine as the initiator. Although the polymerization proceeds through an anionic mechanism, the reaction seems not to be a "living polymer" polymerization, as previously reported for other types of β-lactones, and no correlation has been detected between molecular weight and the initiator/monomer ratio. Only rather low molecular weight polymers were obtained, as was observed previously for the racemic monomer, probably because of as yet unidentified transfer reactions. The resulting PMLA*BE is optically active. The solid polymer is semicrystalline with a complex DTA peak in the 120-160°C temperature range. The broadness of the peak is probably connected to the presence of D-units in the poly-L chains which reduce both the degree of crystallinity and the melting point of the polymer. Nevertheless, the crystallinity of the polymer is high enough that it is insoluble in most of the organic solvents. Only N-methylpyrrolidone, dichloromethane and hexafluoroacetone have been found to be good solvents for that optically active polymer so far.

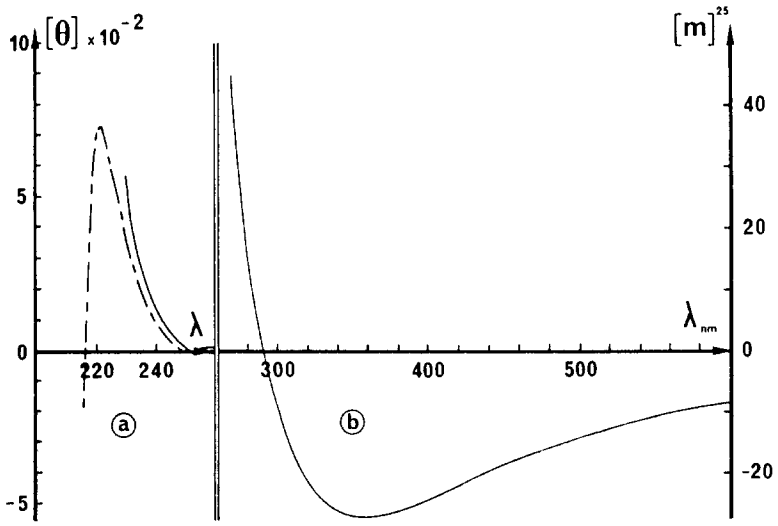


Figure 1 CD (a) and ORD (b) of PMLA*BE. Dichloromethane ———— hexafluoroacetone — - — - — .

Figure 1 shows the ORD curve of PMLA*BE in dichloromethane. This curve is complex, that is it does not obey the one-term DRUDE equation $[\alpha] = A (\lambda^2-\lambda_c^2)$. The positive rotations observed below 380 nm are caused by the presence of a positive CD band located in the range 200-250 nm with a maximum located at λ_{max} = 220 nm, accessible in hexafluoroacetone only. The sharp decrease of ellipticity below 220 nm and the presence of a cross over point at 217 nm suggest that a strong negative CD band is located below 215 nm and is responsible for the negative optical rotations observed in the visible. The ^1L_b band of the aromatic benzyl ester pendant group detected in UV absorption gives rise to a series of very weak CD bands at about 260 nm.

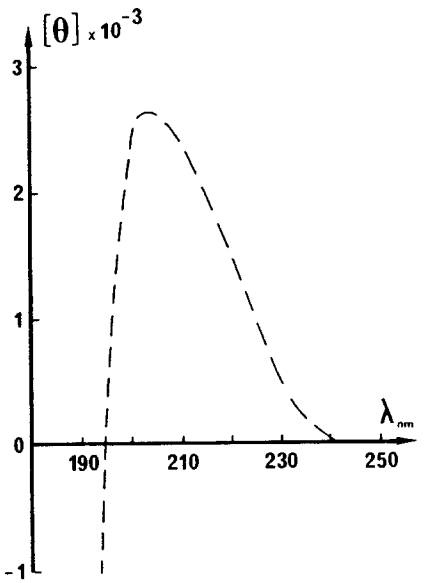


Figure 2 CD spectrum of PMLA*100 Na in salt-free water.

The removal of benzyl protective groups in PMLA*BE can be carried out by catalytic hydrogenolysis and leads to optically active poly(β-malic acid), which is soluble in water at all pH values as its racemic homologue (9). The CD spectrum of the sodium salt, PMLA*100 Na, shows a positive CD band in salt-free water (Figure 2). No aromatic absorption band is detectable by UV, so it is concluded that the positive CD band is due to the presence of optically active ester chromophores in the main chain and/or carboxylate chromophores in side chains.

Since it is now possible to prepare stereoregular optically active PMLA*100, in the future we will take advantage of the presence of the chiral center in the repeating units as a means of easily obtaining stereocopolymers of various configurational compositions.

Acknowlegments

This work was supported by CNRS and NSF through their 1980 and 1982 international cooperative program.

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Accepted August 4, 1985

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