Polydepsipeptides. 6. Synthesis of Sequential Polymers Containing Varying Ratios of L-Alanine and L-Lactic Acid

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ABSTRACT: The synthesis of several new sequential polydepsipeptides has been carried out by the active ester method on a matrix of diatomaceous earth (Celite). The new polymers are poly[(L-Ala)$_2$-L-Lac], poly[(L-Ala)$_3$-L-Lac], poly[(L-Ala)$_2$-(L-Lac)$_2$], poly[(L-Ala)$_3$-(L-Lac)$_2$], and poly[(D-Ala)$_2$-L-Lac] (throughout the manuscript lactic acid is abbreviated Lac; otherwise standard abbreviations are used). The oligodepsipeptide monomers were synthesized by the back-up procedure from the C-terminal Lac residue. Polymer viscosities of 0.15–0.50 dL/g indicate molecular weights of approximately 2–8 × 10^4 daltons. The best general polymerization procedure involved deposition of the trifluoroacetic acid salt of the monomer pentachlorophenyl ester on the Celite followed by drying. Thermal condensation in vacuo required gradual increases in the polymerization temperatures to 150–190 °C. The polymers were obtained in 50–85% yield and were characterized by viscosity, solubility, and NMR spectroscopy. The unique conformational properties of these polymers are of importance in understanding the behavior of homo and sequential polypeptides. In the following paper we describe the conformational analysis of poly[(L-Ala)$_2$-L-Lac]. The conformational studies of the other polymers will be reported in the near future.

Polydepsipeptides are copolymers of α-amino and α-hydroxy carboxylic acids with neighboring monomers linked either by an amide or an ester bond. Because of the close structural similarity of amide and ester groups, polydepsipeptides are particularly appropriate models for the conformational and optical properties of polypeptides and proteins. Since both the amide and ester groups strongly favor the planar trans conformation, the skeletal geometries of peptide and depsipeptide chains are very similar. With the exception of their hydrogen-bonding characteristics, the factors that determine conformational energy of the two types of chains are also closely related. Thus, comparison of the conformational properties of polypeptides and polydepsipeptides will allow assessment of the importance of hydrogen bonding in determining polypeptide chain conformation.

Initial work in this laboratory involved the synthesis of alternating polydepsipeptides composed of L-alanine or L-valine units with the alanine analogue L-lactic acid.[1] Extensive conformational analysis was carried out on poly-L-Ala-L-Lac[2,3] as well as the initial work on poly-L-Val-L-Lac.[4] In the accompanying paper, the conformational behavior of poly[(L-Ala)$_2$-L-Lac] is discussed.[5] The results of these studies indicate a definite relationship between the ratio of alanine to lactic acid groups per repeat unit and the conformational properties. To investigate this effect further and to establish the importance of the sequence of the two types of groups on the conformational behavior, additional polymers were required.

The method of synthesis of these polymers involved active ester "monomers" composed of oligodepsipeptides, e.g., TFA·L-Ala-L-Ala-L-Lac-OPcp where TFA is trifluoroacetic acid and Pcp is pentachlorophenyl. Active ester polymerizations in solution have been extensively employed in the synthesis of sequential polypeptides[6] since their initial development.[7,8] Molecular weights for these polymers are often low, however. Bulk polymerization has been examined for several pentachlorophenol ester monomers and found to give low yields of the desired polymers.[1,9] The use of an inert matrix (Celite diatomaceous earth) to enhance removal of the pentachlorophenol during thermal polymerization in vacuum has led to higher yields and molecular weights for polydepsipeptides.[1] This procedure has further been applied to the synthesis of sequential polypeptides with good results.[10] In all cases where optical purity was determined, the polymers obtained by this method were free from racemization.[1,10] In this paper we describe the synthesis and polymerization of several new depsipeptide monomers containing alanine and lactic acid. The monomers and polymers obtained are characterized by solubility, viscosity, NMR, and optical rotation.

Results

Table I lists the important intermediates in the monomer syntheses along with characteristic physical properties. An interesting relationship is evident between these properties and the number or ratio of Ala and Lac units in an homologous series. For example, in the series Boc-Ala$_n$-Lac-OBzl where n increases from 1 to 3 (compounds **4, 5,** and **7,** Scheme I), the melting point gradually increases while the R_f value decreases and the polarity of the recrystallization solvent increases. These trends may be related to increasing molecular weight and/or to an increasing number of intermolecular hydrogen-bonding units per molecule. In comparing the ratio of alanine and lactic acid per molecule, the melting point increases in the series **9** (Ala/Lac = 0.5) < **10** (1) < Boc-Ala-Lac-Ala-Lac-OBzl[1] (1) < **4** (1) < **5** (2) < **12** (1.5) < **7** (3). While total molecular weight may be influencing the relative positions of **5** and **12,** there seems to be a general trend of increasing melting point with an increasing ratio of Ala to Lac units. Even with these oligomeric depsipeptides, the relative importance of the hydrogen-bonding residues in intermolecular interactions is evident.

In general, the monomer syntheses were straightforward and employed well-established, racemization-free procedures.[1,10] Schemes I and II give syntheses for monomer precursors **8** and **13** as well as the intermediates for **6** and **11.** Because of the clean and essentially quantitative removal of both the Boc and benzyl groups of these compounds, purification and characterization was only carried out on the Boc-benzyl esters and Boc-Pcp esters (Table I). As the molecular weight of the intermediates increased, i.e., from dimer to tri-, tetra-, and pentadepsipeptide, the purification procedures generally became more elaborate. Thus, recrystallization from the reported solvents was generally preceded by column chromatography on silica gel with 1% CH_3OH in $CHCl_3$ using quartz columns and electronic phosphor for UV visualization. Even with chromatography, compounds **7** and **12** were not easily purified and were usually carried on to the Pcp ester after cursory clean-up.

The presence of the Pcp group, of course, greatly facilitates recrystallization from polar solvents, allowing ready purification of the monomer precursors prior to polymerization. Although initial work involved careful purification of the actual monomers (TFA·Ala$_m$-Lac$_n$-OPcp),[1] extensive losses of these hygroscopic materials occurred. In the present work, it

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Table I
Oligodepsipeptide Intermediates and Active Ester Monomer Precursors

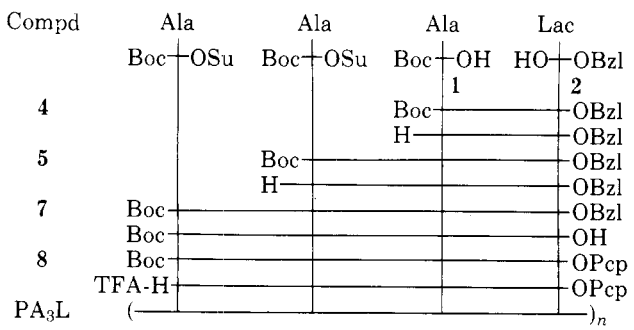
Compd	Mp,[a] °C	Recrystallization solvent	$[\alpha]^{25}_D$, deg (concn in %)[b]	R_f[d]
Boc-Ala-Lac-OBzl (**4**)	82–83	Hexane	−78 (0.2)	0.60
Boc-Ala_2-Lac-OBzl (**5**)	93–94	CCl_4/hexane	−79 (1.0)	0.59
Boc-Ala_2-Lac-OPcp (**6**)	148–150	Dry CH_3OH	−32 (1.0)[c]	0.64
Boc-Ala_3-Lac-OBzl (**7**)	147–149	Purified by column chrom.	−91 (0.12)	0.38
Boc-Ala_3-Lac-OPcp (**8**)		Dry CH_3OH	−75 (1.0)	0.44
Boc-Ala-Lac_2-OBzl (**9**)	Oil		−72 (1.7)	0.62
Boc-Ala_2-Lac_2-OBzl (**10**)	Oil		−81 (0.4)	0.58
Boc-Ala_2-Lac_2-OPcp (**11**)	146–148	Cyclohexane	−81 (0.4)	0.60
Boc-Ala_3-Lac_2-OBzl (**12**)	103–105	Column chrom.	−80 (0.2)	0.41
Boc-Ala_3-Lac_2-OPcp (**13**)	153–155	Column chrom.	−75 (0.8)	0.47
Boc-D-Ala_2-L-Lac-OPcp (**14**)	154–155	Dry CH_3OH	+13 (0.5)[c]	0.62

[a] Uncorrected. [b] CH_3OH as solvent unless otherwise noted. [c] $CHCl_3$. [d] Silica with $CHCl_3$–CH_3OH (95:5).

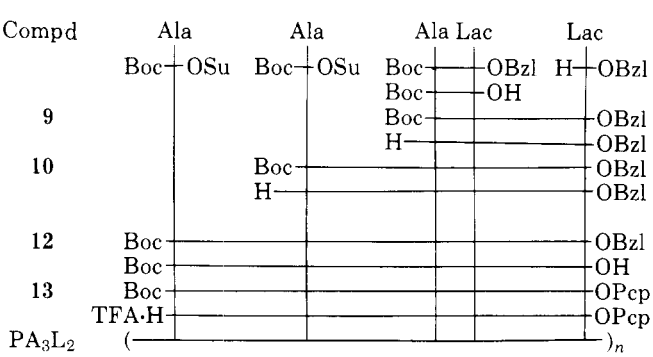
was found that careful purification of the Boc-Ala_m-Lac_n-OPcp intermediates was sufficient. Conversion to the TFA salts and polymerization of these monomers without isolation gave excellent results. All of the polymers listed in Table II were obtained using this procedure.

In the previous synthetic paper in this series,[1] the procedure employed for formation of benzyl L-lactate (**2**) gave relatively low yields. A new procedure has been developed for benzyl ester synthesis involving the highly reactive *N,N'*-diisopropylbenzylisourea prepared from the carbodiimide and benzyl alcohol.[11] This procedure is extremely mild, essentially quantitative, and allows ester formation in the presence of another functionality such as the lactic acid hydroxyl group. However, stronger nucleophiles such as sulfhydryl or amine groups are preferentially alkylated instead of the carboxyl group.[12,13] For hydroxy acids and N-protected amino acids, the use of this reagent is the method of choice.

Scheme I
Synthetic outline for Poly[(L-Ala)$_3$-L-Lac$_2$] and the Intermediate for Poly[(L-Ala)$_2$-L-Lac]



Scheme II
Synthetic Outline for Poly[(L-Ala)$_3$-(L-Lac)] and the Intermediate for Poly[(L-Ala)$_2$-(L-Lac)$_2$]



For the two groups of monomers containing one and two lactic acid residues, the key intermediates are Boc-Ala-Lac-OBzl[1] (**4**) and Boc-Ala-Lac_2-OBzl (**9**), respectively. Both are formed by CDI-mediated coupling of the free acid Boc-Ala-OH (**1**) or Boc-Ala-LacOH with benzyl L-lactate. While **4** is a readily recrystallizable solid, **9** is an oil which is difficult to purify. However, deprotection of the amine of **9** with formation of the hydrochloride followed by ether trituration led to an essentially pure intermediate for synthesis of **10.**

Schemes I and II give the general outlines for the synthesis of poly[(L-Ala)$_3$-L-Lac] and poly[L-Ala)$_3$-(L-Lac)$_2$], respectively, while intermediates **5** and **10** in these schemes were employed in the synthesis of poly[(L-Ala)$_2$-L-Lac] and poly-[L-Ala)$_2$(L-Lac)$_2$]. The structures of these polymers are shown in Figure 1. Other than ester bond formation with the Lac hydroxyl group, all couplings involved DCCI activation mediated directly or indirectly with *N*-hydroxysuccinimide (HOSu). For greatest generality and ease of purification, Boc-Ala-OSu (**3**)[15] was prepared from **1** and HOSu and employed in active ester couplings in water–dioxane mixtures or in THF–CH_2Cl_2. Liberation of the appropriate free amine from its hydrochloride salt in the presence of **3** led to smooth amide bond formation. For the synthesis of **5,** both this procedure and one-step coupling of Boc-AlaOH with HAla-Lac-OBzl in the presence of DCCI and HOSu gave comparable results. However, for the synthesis of higher homologues (**7, 10,** and **12**), use of **3** gave much cleaner product mixtures and higher yields. Thus **3** is the reagent of choice for tetramer and pentamer synthesis in these series.

For the synthesis of poly[L-Ala)$_3$-L-Lac], an alternate monomer was also employed to determine the role of residue sequence on ease of synthesis and polymerization. The intermediate employed was Boc-Ala_2-Lac-Ala-OPcp, which was obtained by procedures analogous to the above. Purifications of intermediates Boc-Ala-Lac-Ala-OBzl and Boc-Ala_2-Lac-Ala-OBzl were difficult and overall yields were low. The polymer obtained has a specific viscosity of 0.12 dL/g and identical NMR and solubility behavior to that from **8.** In addition, the solid state IR spectra of both polymers in ordered and disordered conformations were identical. Thus, the alternative residue sequence in the monomer does not appear to offer any advantages for polymer synthesis. Indeed, using **8** much higher molecular weights were obtained as evidenced by the specific viscosity of 0.50 dL/g.

An alternative synthesis was also employed for **12.** Using the active ester procedure, **3** was condensed with alanine to give Boc-Ala-AlaOH[16] in one step. This compound was activated with DCCI and coupled with HAla-Lac_2-OBzl to give **8.** The physical properties of **8** and the polymer eventually obtained, poly[(L-Ala)$_3$-(L-Lac)$_2$], were identical for both sequences. This alternate sequence, however, was complicated

Table II
Polymerization Conditions and Properties of Sequential Polydepsipeptides Containing L- or D-Alanine [a] and L-Lactic Acid [b]

Polymer	Polymerization temp (h)	^{1}H NMR[d] shifts in δ (ppm) (no. of protons) NH	α-CH Lac	α-CH Ala	CH_3	Mp, °C	$[\alpha]^{27}_D$, deg (concn)[e]	$[\eta]$,[g] dL/g
PA_2L[h]	65 (4), 118 (16)	7.5 (2)	5.3 (1)	4.5 (2)	1.5 (9)			0.34
	65 (4), 118 (16)[c]	7.5 (2)	5.3 (1)	4.5 (2)	1.5 (9)	265 dec	−89 (0.2)	0.17
							−145 (0.2)[f]	
PDA_2L[h]	125 (18), 150 (5)	7.5 (2)	5.3 (1)	4.5 (2)	1.5 (9)	265 dec	+78 (0.2)[f]	0.15
PA_3L[h]	125 (18), 150 (5)	7.6 (3)	5.3 (1)	4.5 (3)	1.5 (12)	275 dec	−60 (0.5)	0.19
	110 (18), 170 (18)	7.6 (3)	5.3 (1)	4.5 (3)	1.5 (12)			0.21
	150 (5), 190 (18)	7.6 (3)	5.3 (1)	4.5 (3)	1.5 (12)	>300		0.50
PA_2L_2[h]	130 (24)	7.4 (2)	5.1 (2)	4.5 (2)	1.5 (12)	215 dec	−79 (0.3)	0.12
	125 (18), 150 (5)	7.4 (2)	5.1 (2)	4.5 (2)	1.5 (12)	215 dec		0.31
PA_3L_2[h]	130 (24)	7.5 (3)	5.2 (2)	4.5 (3)	1.5 (15)	250 dec	−95 (1.0)	0.17
	150 (18), 190 (5)	7.5 (3)	5.2 (2)	4.5 (3)	1.5 (15)	250 dec		0.17

[a] PDA_2L is the only polymer containing D-Ala. [b] Polymers isolated from Celite by extraction with TFE or TFA. [c] On 50 g of monomer. [d] Broad multiplets. [e] % concentration in TFE. [f] In HFIP. [g] In dichloroacetic acid at 25 °C. [h] Poly[(L-Ala)$_2$-L-Lac] (PA_2L); poly[(D-Ala)$_2$-L-Lac] (PDA_2L); poly[(L-Ala)$_3$-L-Lac] (PA_3L); poly[(L-Ala)$_2$-(L-Lac)$_2$] (PA_2L_2); poly[(L-Ala)$_3$-(L-Lac)$_2$] (PA_3L_2).

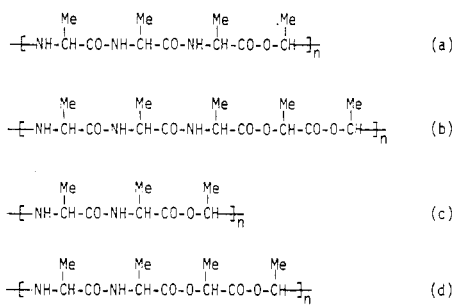


Figure 1. Structures of depsipeptides containing alanine and lactic acid: (a) poly[(L-Ala)$_3$-L-Lac)]; (b) poly[(L-Ala)$_3$-(L-Lac)$_2$)]; (c) poly[L-Ala)$_2$-L-Lac)]; and (d) poly[(L-Ala)$_2$-(L-Lac)$_2$)].

by formation of the hydrate of Boc-Ala-AlaOH. The hydrate was obtained as a result of the reaction conditions (dioxane–water) used and could be dehydrated only with great difficulty. On exposure to air, rehydration of the anhydrous compound and formation of an oil were very rapid. The procedure outlined in Scheme II was cleaner and gave higher overall yields of the desired intermediate.

Table II lists the various polydepsipeptides together with polymerization conditions and selected physical properties. Three types of polymerization procedures were examined for obtaining these polymers: solution, bulk, and matrix. In all cases examined, solution polymerizations of the TFA salts of the Pcp esters in the presence of purified $(n\text{-Bu})_3N$ or Et_3N and dried solvents gave low molecular weight ($[\eta] < 0.1$ dL/g). Several bulk polymerizations were carried out by coating a 500-mL round-bottomed flask with 0.1–0.2 g of monomer and heating in vacuo. Although polymers with higher molecular weights ($[\eta] = 0.14$–0.21 dL/g) could be obtained by this latter procedure, degradation reactions led to brown or dark brown products. The Celite matrix procedure routinely gave good yields of higher molecular weight polymers which were light tan to white in color and gave no evidence of side reactions or degradation. All of the polymers in Table II were obtained using this procedure.

Several factors are important for obtaining good yields of high molecular weight products. First, repeated reprecipitation of the actual monomers from $CHCl_3$ and CH_2Cl_2 by rotary evaporation both after TFA treatment and on Celite deposition is necessary to remove residual TFA. Second, removal of air, water, and solvent from the matrix prior to polymerization by drying in vacuo for at least 18 h increases the molecular weight and decreases discoloration. Third, a gradual or stepwise increase in polymerization temperature is desirable to reduce monomer sublimation and decomposition. In general, higher polymerization temperatures, especially in the final stages of polymerization, resulted in higher molecular weight. This is especially true for higher melting polymers.

In general, higher polymerization temperatures were required to give high molecular weights for the polymers listed in Table II compared to polydepsipeptides[1] and polypeptides[10] prepared previously by this method. Furthermore, the two-stage procedure with increased temperatures often gave polymers with higher intrinsic viscosities even for lower melting polymers poly[(L-Ala)$_2$-(L-Lac)$_2$] and poly[(D-Ala)$_2$-L-Lac]. It should be noted that the melting points of these polymers are much higher than those of the alternating polydepsipeptides poly(L-Ala-L-Lac) (142 °C) or poly-L-valine-L-lactic acid (165 °C). This may be due to increases in the relative number of Ala to Lac residues per repeat unit or to the existence of an ordered conformation in the solid state, i.e., α helix or β structure.

The NMR spectra of the polydepsipeptides all exhibit chemical shifts and integration values consistent with previous polymers and with the relative numbers of hydrogen atoms per functional group. TFA was chosen as the NMR solvent because of the insolubility of one or more of the polymers in other common solvents. Despite expectations based on the ready solubility of poly(L-Ala-L-Lac) in common organic solvents,[2,3] the polymers described here were not soluble in solvents such as dioxane, alkyl alcohols, DMF, or Me_2SO. While several of the polymers could be dissolved in chloroform, prior dissolution in TFE was required. The best general solvents were halogenated acids and alcohols. All of the polymers were soluble in dichloroacetic acid, TFA, hexafluoroacetone sesquihydrate, and hexafluoro-2-propanol and all but poly[D-Ala)$_2$-L-Lac] in TFE and TFE–$CHCl_3$ mixtures. An explanation for the insolubility of poly[(D-Ala)$_2$-L-Lac] in TFE or TFE–$CHCl_3$ is not readily apparent, since even poly-L-alanine is somewhat soluble in the latter solvent mixture.

The specific rotations given are consistent with rotations observed for the intermediates. Differences may well be due to varying amounts of ordered or disordered conformations. Complete investigation of the circular dichroism of these polymers is under way and the conformational behavior of these polymers will be described in detail shortly.

In general, good yields (50–85%) of relatively high molecular weight polymers were obtained with the desired sequences of

Ala to Lac per repeat unit. The use of TFA salts of Pcp esters with the Celite matrix was the method of choice for these polymers. Higher polymerization temperature, especially with a stepwise increase, gave higher molecular weights and cleaner polymers.

Discussion

The general procedure employed for monomer synthesis, the "back-up" technique, has been demonstrated to be racemization free for both depsipeptide[1] and peptide[10] oligomers. Furthermore, the polymers obtained from these oligomers were also shown to be racemization free. This polymerization procedure, involving the thermal condensation in vacuo of TFA salts of Pcp esters deposited on Celite, gives high molecular weight polymers which are essentially optically pure. Polymers obtained by this method are ideal for conformational studies where optical purity is necessary. Furthermore, this method is essential for production of high molecular weight sequential polymers containing known repeat units which cannot be synthesized by the random copolymerization of the corresponding α-amino acid *N*-carboxyanhydrides and α-hydroxy acid anhydrosulfites.[14] The adaptability of this procedure to large scale synthesis was demonstrated by production of over 50 g of poly[(L-Ala)$_2$-L-Lac] in a single batch.

In both synthetic schemes used (I and II), the lactic acid residue was incorporated at the carboxyl terminus for several reasons. First, formation of the ester bond involving the lactic acid hydroxyl was generally a slower process with lower yields than amide bond formation. The low-yield step in the first coupling was therefore the least expensive in terms of overall yield. Second, it was hoped that the penultimate ester group would further reduce the possibility of racemization during active ester synthesis and thermal polymerization. More importantly, it was feared that with the relatively high polymerization temperatures employed for the tetra- and pentadepsipeptides, the intramolecular attack of the terminal amine to give cyclic dipeptides might compete with intermolecular attack. Moving the more reactive ester groups as far from the amine terminus as possible minimizes this possible side reaction. Finally, positioning the lactic acid group(s) at the carboxyl terminus keeps the syntheses as general as possible for the desired homologous series of polymers.

The polymers chosen for synthesis were designed to cover a range of ratios of Ala to Lac residues and to examine the importance of their relative position within the repeating sequence. Both considerations are ultimately concerned with the type of ordered and disordered conformations available to the polymers and the relative stability of the ordered structures. Poly-L-Ala can adopt an α helix, an antiparallel β structure, or a random coil in the solid state and in solution.[17] Poly(L-Ala-L-Lac) apparently does not adopt the α-helical conformation in the solvents examined to date or in the solid state.[3] For this polymer, the α helix would require formation of intramolecular hydrogen bonds between the amide hydrogen atoms of the alanine residue and the carbonyl oxygen atom of the ester group, three residues away; the carbonyl oxygen atom of the amide group would not be hydrogen bonded.[2] Other conformations are evidently more favorable in this case.

Poly[Ala$_2$-Lac] was synthesized with the goal of increasing the relative number of hydrogen bonds possible per repeat unit. In addition, the sequence of the trimeric repeat unit in the α helix allows formation of the more favorable intramolecular hydrogen bonds between an amide hydrogen atom and the amide carbonyl oxygen atom three residues away. The number and sequence of hydrogen-bonding groups for poly[(L-Ala)$_2$-L-Lac] favors the α helix in helix-supporting solvents.[5]

Two of the remaining polymers fall into the series of the gradually increasing ratio of potential hydrogen-bonding groups per repeat sequence. The series includes poly(L-Ala-L-Lac) (Ala/Lac = 1.0), poly[(L-Ala)$_3$-(L-Lac$_2$)] (1.5), poly[(L-Ala)$_2$-L-Lac] (2.0), and poly[(L-Ala)$_3$-L-Lac] (3.0). Qualitatively, the relative tendency of poly[(L-Ala)$_3$-(L-Lac)$_2$] to assume the α-helical conformation should be intermediate between poly(L-Ala-L-Lac) and poly[(L-Ala)$_2$-L-Lac]. To examine the hydrogen-bonding behavior of the ester carbonyl in a strongly α-helical polymer, poly[(L-Ala)$_3$-L-Lac] was included.

The behavior of poly(L-Ala-L-Lac) prompted us to examine an alternative repeat unit sequence with poly[(L-Ala)$_2$-(L-Lac)$_2$]. Although the same relative number of Ala to Lac residues is maintained, this polymer has the potential for formation of an α helix in which one amide carbonyl and one ester carbonyl group can engage in intramolecular hydrogen bonding with the two amide hydrogen atoms. Furthermore, poly[(L-Ala)$_2$-(L-Lac)$_2$] is the next lower homologue of poly[(L-Ala)$_3$-(L-Lac)$_2$] and a study of the conformational behavior of these two polydepsipeptides should allow qualitative evaluation of the helix-disrupting ability of two adjacent Lac residues.

Finally the behavior of poly[(L-Ala)$_2$-L-Lac] indicates a somewhat surprising mobility for the ester carbonyl in the α-helical conformation.[5] To further explore the amount of mobility which may be accommodated in this polymer, the analogue containing the enantiomeric Lac residue would be required. It was found to be more practical to synthesize poly[(D-Ala)$_2$-L-Lac], however, in which two D-alanine residues and a L-lactic acid moiety satisfy the same stereochemical requirements as two L-alanine residues plus D-lactic acid. A left-handed α helix is expected for this polymer, similar to the right handed one of poly[(L-Ala)$_2$-L-Lac], if sufficient flexibility exists in this conformation for incorporation of the Lac residue of opposite configuration.

A detailed conformational analysis of poly[(L-Ala)$_2$-L-Lac] is presented in the accompanying paper.[5] Similar analyses are under way on the remaining polymers described here. These studies will be presented in the near future. In the following paper we present conformational analysis of poly[(L-Ala)$_2$-L-Lac]. In subsequent manuscripts we will report on the conformations of the other polymers synthesized.

Experimental Section

t-Boc-L-alanine (1) and *t*-Boc-D-alanine were purchased from Bachem. Carbonyldimidazole (CDI) was prepared freshly prior to use.[18] Dicyclohexylcarbodiimide (DCCI) was distilled and stored at 5 °C. *N*-Hydroxysuccinimide (HOSu) and pentachlorophenol (Pcp) were recrystallyzed before use. All solvents were dried according to literature methods and stored over 4 Å molecular sieves. The Celite was washed with 4 N HCl, TFA, and then with water until a neutral filtrate was obtained. This was followed by washing with TFE, ethanol, and finally ethyl ether before the Celite was dried in vacuo at 120 °C for 48 h. NMR chemical shifts are δ (ppm); IR values are in cm^{-1}. Microanalyses were performed by Galbraith Laboratories, Inc. NMR spectra were obtained using a Varian T-60 spectrometer and optical rotations were measured on a Perkin-Elmer polarimeter 141. All melting points are uncorrected.

***N,N'*-Diisopropyl-*O*-benzylisourea.** Benzyl alcohol (147.0 g, 1.36 mol) was added with stirring to a mixture of cuprous chloride (0.5 g) in *N,N'*-diisopropylcarbodiimide (172.3 g, 1.36 mol) over a 30-minute period at 0 °C. After an additional 1 h at 0 °C, the reaction was greater than 95% complete as evidenced by disappearance of the infrared band at 2100 cm^{-1} (diimide) and the appearance of the band at 1660 cm^{-1} (isourea). The mixture was stirred at room temperature for 17 h to ensure complete reaction. The volume was then doubled with hexane and the solution was applied to a filter pad of neutral alumina to remove copper salts. The product was eluted with a total volume of 1.7 L of hexane, at which time the IR spectrum indicated that all the benzylisourea had been removed from the column. The solvent was evaporated under reduced pressure, and the resulting oil was

pumped under high vacuum for 24 h. This product was of sufficient purity to be used directly for ester formation. Distillation may be carried out at 105 °C under 1.1 mmHg vacuum; IR (neat) 3140 (w), 1660 (s); NMR ($CDCl_3$) δ 1.1 (d, 6 H), 3.2 (m, 1 H), 3.8 (m, 1 H), 5.1 (s, 2 H), 7.2 (s, 5 H).

Benzyl-L-lactic Acid (2). L-Lactic acid (9 g, 100 mmol) was added to *N,N'*-diisopropylbenzylisourea (23.3 g, 100 mmol) with stirring. The mixture became very viscous within 10 min and was stirred intermittently for 1 h. The volume was then increased to 200 mL with THF, and the mixture was stirred for 48 h at room temperature. After cooling the mixture to −15 °C, the diisopropylurea was removed by filtration and the THF was evaporated under reduced pressure. The resulting thick oil was pumped under high vacuum for 24 h to give benzyl lactate (17.4 g, 95%) which was used without further purification: IR (neat) 3450 (b), 1740; NMR ($CDCl_3$) δ 1.35 (d, 3 H), 3.2 (b, 1 H), 4.2 (q, 1 H), 5.15 (s, 2 H), 7.25 (s, 5 H).

***tert*-Butyloxycarbonyl-L-alanyl-L-lactic Acid Benzyl Ester (4).** A solution of *tert*-butyloxycarbonyl-L-alanine (25.0 g, 0.13 mol) in dry methylene chloride (100 mL) was cooled to 0 °C in an ice bath with stirring and treated with a solution of carbonyldiimidazole (20.6 g, 0.125 mol) in methylene chloride (150 mL) over 30 min. After stirring for an additional 20 min at 0 °C, benzyl L-lactate (23.7 g, 0.132 mol) was added over a 30-min period. The reaction mixture was maintained at 0 °C for a further 2 h and then at room temperature for 3 days. The mixture was evaporated to a thick oil which was dissolved in ether (220 mL). This solution was extracted with H_2O, citric acid, saturated $NaHCO_3$, and H_2O and dried over magnesium sulfate. The magnesium sulfate was removed by filtration, and the filtrate was evaporated in vacuo to yield a thick crystalline mass. Recrystallization from hexane (80 mL) and drying in vacuo gave *tert*-butyloxycarbonyl-L-alanyl-L-lactic acid benzyl ester (31.5 g, 68%): mp 82–83 °C (lit.[1] mp 82–83 °C); IR ($CHCl_3$) 1760 (b), 1690; NMR ($CDCl_3$) δ 1.45 (s, 9 H), 1.4 (d, 3 H), 1.5 (d, 3 H), 4.4 (m, 1 H), 5.2 (q, 1 H), 5.2 (s, 2 H), 7.35 (s, 5 H).

***tert*-Butyloxycarbonyl-L-alanyl-L-alanyl-L-lactic Acid Benzyl Ester (5).** *tert*-Butyloxycarbonyl-L-alanyl-L-lactic acid benzyl ester (22.8 g, 0.065 mol) was deprotected to L-alanyl-L-lactic acid benzyl ester hydrochloride by stirring for 1 h in 4 N HCl in dioxane (40 mL) followed by precipitation from hexane. Filtering and drying in vacuo gave pure material, mp 159–161 °C (lit.[1] mp 150–153 °C).

The above salt (18.7 g, 0.065 mol) was dissolved in dry DMF (120 mL) together with *N*-methylmorpholine (6.5 g, 0.065 mol), *N*-hydroxysuccinimide (7.5 g, 0.065 mol), and *t*-Boc-L-alanine (12.0 g, 0.065 mol). The reaction mixture was cooled to −20 °C and a solution of dicyclohexylcarbodiimide (13.1 g, 0.065 mol) in dry DMF (70 mL) was added over 45 min. The temperature was maintained at −20 °C for 2 h and the mixture was then warmed to room temperature and stirred for 48 h. Acetic acid (5 mL) was added and after 20 min the dicyclohexylurea was removed by filtration. The solution was evaporated in vacuo to a thick syrup which was dissolved in ethyl acetate (200 mL) and filtered to remove dicyclohexylurea. The filtrate was washed thoroughly with H_2O, saturated $NaHCO_3$, citric acid, and again with H_2O and dried over magnesium sulfate. Filtration and evaporation gave an oil which was dissolved in a minimum amount of $CHCl_3$ and passed through a bed of neutral alumina. Elution with $CHCl_3$ was continued until the IR spectrum indicated that no further product was being eluted (total volume, 200 mL). The solvent was removed in vacuo and the resulting thick oil was pumped under high vacuum for 24 h to give compound **5** (29 g, 100%). The NMR and IR spectra indicated no detectable impurities. The oil was used for subsequent steps without further purification. Alternatively, trituration with water gave a solid: mp 89–92 °C; IR (neat) 3320, 1750, 1690 (sh), 1660, 1525; NMR ($CDCl_3$) δ 1.4 (m, 18 H), 4.15 (m, 1 H), 4.6 (m, 1 H), 5.2 (q, 1 H) 5.15 (s, 1 H), 7.3 (s, 5 H).

An alternative synthesis of **5** involves prior formation and recrystallization of Boc-Ala-OSu according to the procedure of Anderson et al.[14] Thus, a solution of Boc-Ala-OSu (0.99 g, 3.5 mmol) in dioxane (20 mL) was added dropwise to a mixture of Ala-Lac-OBzl·HCl (1.0 g, 3.5 mmol) and $NaHCO_3$ (0.9 g, 10.5 mmol) in water (20 mL). The mixture was stirred overnight and the dioxane was then removed in vacuo. The aqueous suspension was extracted twice with ethyl acetate and the combined extracts were washed with aqueous solutions of citric acid, $NaHCO_3$, and NaCl. Evaporation of the solvent, trituration with water, and drying in vacuo gave the pure white product in comparable yields to the above procedure.

***tert*-Butyloxycarbonyl-L-alanyl-L-alanyl-L-lactic Acid Pentachlorophenyl Ester (6).** Boc-Ala-Ala-Lac-OBzl (27.4 g, 0.065 mol) was dissolved in dry tetrahydrofuran (182 mL) and 0.7 g of 10% palladium on charcoal was added. This mixture was hydrogenated at 60 psi for several hours, the reaction mixture was filtered through Celite, and the solvent was removed in vacuo. The resulting thick oil was dried under high vacuum for 24 h to give Boc-Ala-Ala-Lac-OH: NMR ($CDCl_3$) δ 1.4 (s + doublets, 18 H), 4.1 (m, 1 H), 4 (m, 2 H), 5.2 (q, 1 H), 7.8 (s, 1 H).

Boc-Ala-Ala-Lac-OH (19.6 g, 0.059 mol) was dissolved in dry DMF (120 mL) and the volume reduced to 100 mL by evaporation in vacuo to ensure dryness and removal of lower boiling contaminants such as acetic acid. The solution was then cooled to 0 °C in an ice bath and a solution of dicyclohexylcarbodiimide (12.1 g, 0.059 mol) in DMF (60 mL) was added with stirring. After 1 h, pentachlorophenol (15.6 g, 0.059 mol) in DMF (60 mL) was added dropwise over a period of 1 h, and stirring was continued an additional 2 h at 0 °C and 48 h at room temperature. Acetic acid (6 mL) was added to destroy any remaining carbodiimide and after 30 min the dicyclohexylurea was filtered off. The filtrate was evaporated in vacuo and the resulting mass was dissolved in ethyl acetate, filtered, washed as for compound **5** above, and dried over magnesium sulfate. Filtration and evaporation under reduced pressure gave a crystalline mass which was dissolved in a minimum of chloroform and passed through a short silica column. Elution with chloroform was continued until the IR spectrum indicated that no more product was being eluted. Evaporation of the solvent under reduced pressure gave a crystalline mass which was recrystallized from dry methanol to give Boc-Ala-Ala-Lac-OPcp (23.5 g, 69%): mp 148–150 °C; IR (KBr) 3340, 1790, 1755, 1685, 1660; NMR ($CDCl_3$) δ 1.33 (d, 3 H), 1.40 (s, 9 H), 1.45 (d, 3 H), 1.75 (d, 3 H), 4.2 (m, 1 H), 5.5 (q, 1 H).

***tert*-Butyloxycarbonyl-L-alanyl-L-alanyl-L-alanyl-L-lactic Acid Benzyl Ester (7).** Triethylamine (2.36 g, 0.023 mol) was added to a cooled (0 °C) solution of L-alanyl-L-alanyl-L-lactic acid benzyl ester hydrochloride (4.2 g, 0.0117 mol) in dry CH_2Cl_2 (50 mL). A solution of *tert*-butyloxycarbonyl-L-alanine *N*-hydroxysuccinimide ester (3.35 g, 0.0117 mol) in CH_2Cl_2 (150 mL) was added dropwise over a 1-h period. Stirring was continued at 0 °C for 2 h and at 25 °C for 18 h. The mixture was then cooled to −15 °C and the triethylamine hydrochloride was removed by filtration. The filtrate was washed with aqueous solutions of citric acid, $NaHCO_3$, and NaCl (saturated) and the solvent was evaporated in vacuo. The product was dried overnight in vacuo to give a white powder (4.2 g, 73%): mp 143–145 °C; NMR ($CDCl_3$) δ 1.25–1.55 (overlapping d, 12 H), 1.45 (s, 9 H), 4.15 (q, 1 H), 4.35 (m, 2 H), 5.10 (s, 2 H), 5.13 (m, 2 H), 6.83 (d, 2 H), 7.28 (s, 5 H).

***tert*-Butyloxycarbonyl-L-alanyl-L-alanyl-L-alanyl-L-lactic Acid Pentachlorophenyl Ester (8).** The benzyl ester **7** was converted to the free acid by hydrogenation of a THF solution for 3 h at 50 psi over 10% palladium on carbon.The catalyst was removed by filtration through a Celite filter pad and the filtrate was evaporated in vacuo to give the acid quantitatively.

tert-Butyloxycarbonyl-L-alanyl-L-alanyl-L-alanyl-L-lactic acid (3.21 g, 7.96 mmol) was dissolved in dry THF (50 mL) and that solution was cooled to 0 °C. A solution of DCCI (1.64 g, 8 mmol) in THF (10 mL) was added dropwise and stirring was continued for 30 min after complete addition. A solution of pentachlorophenol (2.12 g, 8 mmol) in THF (25 mL) was then added dropwise over a 30-min period and stirring was continued for 2 h at 0 °C and 18 h at 25 °C. A few drops of glacial acetic acid were added and after 30 min the solution was filtered to remove dicyclohexylurea (DCU). Evaporation of the solvent in vacuo gave less than one-fourth of the expected amount of product. Repeated extraction of the THF-insoluble material (containing DCU) with methylene chloride led to recovery of most of the product which was found to be only slightly soluble in THF and ethyl acetate. Recrystallization from dry methanol gave white needles (2.5 g, 48%). A second recrystallization gave TLC pure material: mp 205–206 °C; NMR ($CDCl_3$) δ 1.27–1.58 (d's, 9 H), 1.45 (s, 9 H), 1.75 (d, 3 H), 4.26 (p, 1 H), 4.72 (m, 2 H), 5.25 (d, 1 H), 5.65 (q, 1 H), 7.15 (m, 2 H). Anal. Calcd for $C_{23}H_{28}N_3O_8Cl_5$: C, 42.36, H, 4.33; N, 6.44. Found: C, 42.20; H, 4.48; N, 6.38.

***tert*-Butyloxycarbonyl-L-alanyl-L-lactyl-L-lactic Acid Benzyl Ester (9).** The benzyl ester **4** was converted to the free acid in the same manner as described for the benzyl ester **5**. The acid (7.9 g, 0.03 mol) was dissolved in dry CH_2Cl_2 (50 mL) and cooled to 0 °C. Activation with CDI and condensation with 1 equiv of benzyl L-lactate for 7 days (analogous to **4**) gave compound **9** (8.2 g, 65%) as a clear, viscous oil. This material contained 5–10% unreacted benzyl L-lactate which was removed in the next step: NMR ($CDCl_3$) δ 1.42 (d, 6 H), 1.45 (s, 9 H), 1.53 (d, 3 H), 4.28 (p, 1 H), 5.05 (m, 3 H), 5.15 (s, 2 H), 7.25 (s, 5 H).

***tert*-Butyloxycarbonyl-L-alanyl-L-alanyl-L-lactyl-L-lactic Acid Benzyl Ester (10).** Impure compound **9** was treated with 4 N HCl in dioxane for 1 h and the clear solution was poured into 20 vol-

umes of hexane. After stirring for 2 h, the viscous oil was allowed to settle and the hexane was decanted. Trituration with dry ether gave a clear, viscous oil free from benzyl L-lactate. This oil was dried in vacuo and used without further purification.

A solution of the above hydrochloride salt (3.3 g, 0.009 mol) in aqueous $NaHCO_3$ (100 mL) was treated dropwise with a solution of Boc-Ala-OSu (2.57 g, 0.009 mol) in dioxane (80 mL). After stirring overnight at 25 °C, the aqueous dioxane was removed in vacuo. The viscous residue was partially dissolved in ether (100 mL) and the suspension was washed with aqueous citric acid, $NaHCO_3$, and saturated NaCl. The solvent was evaporated under reduced pressure and the resulting viscous oil was dried in vacuo for 48 h to give analytically pure product (3.27 g, 74%): NMR ($CDCl_3$) δ 1.35–1.55 (d's, 12 H), 1.45 (s, 9 H), 4.08 (p, 1 H), 4.35 (p, 1 H), 5.00 (m, 3 H), 5.10 (s, 2 H), 6.80 (d, 1 H), 7.20 (s, 5 H). Anal. Calcd for $C_{24}H_{34}N_2O_9$: C, 58.29; H, 6.83; N, 5.66. Found: C, 57.66; H, 6.81; N, 4.98.

***tert*-Butyloxycarbonyl-L-alanyl-L-alanyl-L-lactyl-L-lactic Acid Pentachlorophenyl Ester (11).** The debenzylation of **10** was carried out in the same manner as that described for **5** to give the very hygroscopic, low-melting acid quantitatively. This acid (2.4 g, 0.006 mol) was converted to the Pcp ester by the same procedure used for **6** to give impure product (2.52 g, 65%) as a low melting glass. Purification was achieved in two parts. Initial recrystallization from hot cyclohexane gave needles contaminated with pentachlorophenol which are only slightly soluble in cyclohexane. Extraction of these crystals with hot cyclohexane resulted in a white powder which was analytically pure: mp 146–148 °C; NMR ($CDCl_3$) δ 1.38–1.58 (d's, 9 H), 1.42 (s, 9 H), 1.75 (d, 3 H), 4.18 (p, 1 H), 4.50 (p, 1 H), 4.80 (d, 1 H), 5.05 (q, 1 H), 5.35 (q, 1 H), 6.92 (d, 1 H). Anal. Calcd for $C_{23}H_{27}N_2O_9Cl_5$: C, 42.31; H, 4.17; N, 4.29. Found: C, 42.59; H, 4.20; N, 4.13.

***tert*-Butyloxycarbonyl-L-alanyl-L-alanyl-L-alanyl-L-lactyl-L-lactic Acid Benzyl Ester (12).** The same procedure described for the synthesis of **10** was employed to give, first, the hygroscopic hydrochloride salt (1.73 g, 0.0047 mol) and, then, the coupled product (2.2 g, 82%). This material could not be purified by chromatography or recrystallization and was carried on to the next step.

***tert*-Butyloxycarbonyl-L-alanyl-L-alanyl-L-alanyl-L-lactyl-L-lactic Acid Pentachlorophenyl Ester (13).** Using the same procedure as described for **11**, the hygroscopic free acid (1.67 g, 0.0035 mol) was converted to impure product (1.6 g, 63%), which was purified by column chromatography on silica gel with 1% CH_3OH in $CHCl_3$: mp 153–155 °C; NMR ($CDCl_3$) δ 1.30–1.55 (d's, 12 H), 1.45 (s, 9 H), 1.75 (d, 3 H), 4.10 (p, 1 H), 4.45 (m, 2 H), 4.95 (d, 1 H), 5.12 (q, 1 H), 5.42 (q, 1 H), 6.63 (d, 2 H). Anal. Calcd for $C_{26}H_{32}N_3O_{10}Cl_5$: C, 43.13; H, 4.46; N, 5.80. Found: C, 42.52; H, 4.63; N, 5.55.

Polymerization Procedure. The Boc-protected pentachlorophenyl ester (200–300 mg, 0.3–0.5 mmol) was stirred for 1 h with TFA (5–10 mL). Excess TFA was evaporated in vacuo to give a clear oil which was dissolved three times in 50 mL of CH_2Cl_2 or $CHCl_3$; the solvent was evaporated in vacuo. The white power or crystals obtained by this procedure were dissolved in CH_2Cl_2 or $CHCl_3$ (50 mL), 300–400 mg of Celite was added, the mixture was stirred for a few minutes, and the solvent was then removed in vacuo. This deposition procedure was repeated twice more. The resulting monomer-coated Celite was dried in vacuo 18–24 h at 25 °C in a suitable polymerization vessel such as a small sublimator, a polymerization tube, or a round-bottom flask fitted with vacuum take-off. (A cold finger is not necessary since pentachlorophenol will sublime to any nonheated surface.) The polymerization vessel was evacuated to 0.1–0.5 mmHg vacuum and placed in an oil bath, the temperature of which was gradually raised over several hours to the appropriate polymerization temperature. The bath was maintained at this temperature for 18 h then raised to the final temperature for 5 h. After cooling, the polymer-coated Celite was removed from vacuum and extracted with an appropriate solvent. Evaporation of the solvent or reprecipitation into ether, filtering, and drying in vacuo gave the white or off-white polymer in 50–85% yield.

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