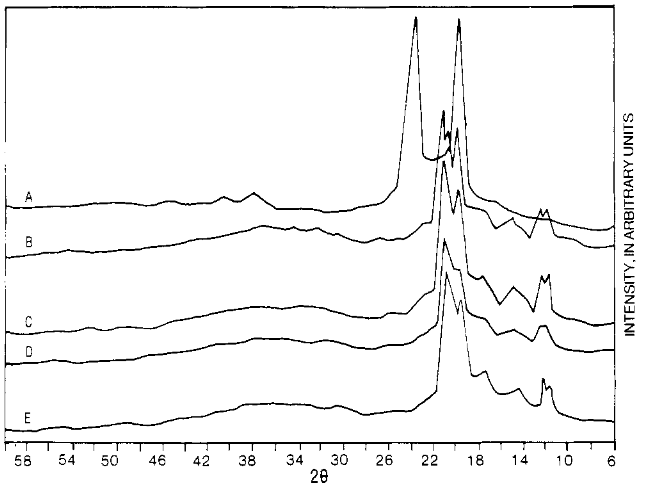

Figure 8. WAXD patterns of (A) linear oligomer of ε-caprolactam, DP = 18, (B) linear oligomer DP = 18/perfluoroglutaric acid adduct, (C) linear oligomer DP = 26/perfluoroglutaric acid adduct, (D) linear oligomer DP = 36/perfluoroglutaric acid adduct, and (E) linear oligomer DP = 75/perfluoroglutaric acid and adduct.

enough to precipitate, the oligomer with DP = 18 formed some, the one with DP = 26 formed more, and the higher oligomeric adducts precipitated completely.

As was mentioned above, intrinsic viscosities of high molecular weight aliphatic polyamides showed no significant reduction upon regeneration from the adducts. This indicates that in this case all macromolecules participated in the formation of adducts, with no segregation according to molecular weight. Because of the stoichiometry of the system, measured by titration, a simple occlusion of low molecular weight macromolecules in the precipitating adduct aggregates is unacceptable. A hypothetical explanation may be that the high molecular weight macromolecules in the polydisperse systems at hand serve with their associated diacid molecules as a matrix along which the lower molecular weight macromolecules (or oligomers) organize themselves. A similar capability of self-organization of oligomers along higher molecular weight molecules was recently reported in the literature.[7,8]

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Polydepsipeptides. 9. Synthesis of Sequential Polymers Containing Some Amino Acids Having Polar Side Chains and (*S*)-Lactic Acid

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ABSTRACT: Sequential polydepsipeptides having polar side chains were prepared by polycondensation of the active ester of the oligodepsipeptides. The N-protected oligodepsipeptides having polar side chains were synthesized by a new method using (*o*-nitrophenyl)sulfenyl *N*-carboxyl-α-amino acid anhydrides (Nps-NCAs) which were shown to react rapidly with the hydroxy group of (*S*)-lactic acid. The polymerization was carried out both by methods involving matrix-mediated thermal reactions and by condensation reactions in solution. Of the two, the solution polymerization yielded polymers in higher yields with high molecular masses ((2.3-12.9) × 10^4 daltons).

Introduction

Recent studies of polydepsipeptides have shown that these molecules exhibit conformational characteristics similar to corresponding polypeptides. Helix-to-coil transitions can be induced in these polymers both thermally and by changes of solvent.[2] Therefore, these macromolecules can serve as good model systems for the study of the helical structures of proteins and are specially well suited to study the role of hydrogen bonding in the stabilization of the helix.

To date our work has concentrated on amino acids and hydroxy acids with alkyl side chains (L- and D-alanine with (*S*)-lactic acid).[3,4] In this paper, we describe the first successful synthesis of polydepsipeptides having protected polar side chains. We have introduced a new method for the synthesis of the depsipeptide "monomers", which in-

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volves the reaction of (*o*-nitrophenyl)sulfenyl *N*-carboxyanhydrides (Nps-NCAs) of α-amino acids with lactic acid.

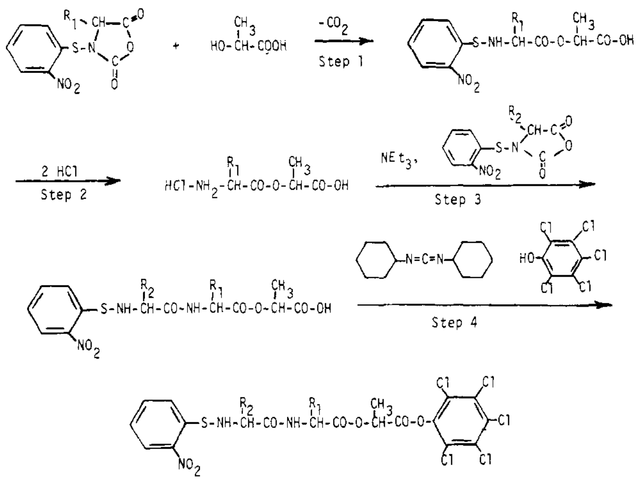
Results

In previous research,[3,4] we have shown that depsipeptide bond formation between amino acids containing nonpolar side chains and lactic acid can be achieved by *N,N'*-carbonyldiimidazole (CDI) mediated coupling of the carboxylic acid group of the N-protected amino acids with the hydroxyl group of lactic acid benzyl ester. The benzyl ester could, in turn, be removed at a later step by catalytic hydrogenation. This synthetic procedure using CDI coupling and the benzyl ester protection of lactic acid cannot be utilized in the synthesis of polydepsipeptides containing glutamic acid and lysine, whose polar side chains are protected by benzyl ester and benzyloxycarbonyl. These groups are removed simultaneously in the process of deprotection of the C-terminal benzyl ester of the main chain by hydrogenation. Thus, in our synthesis of polydepsipeptides with amino acids having protected polar side chains with the benzyl ester and benzyloxycarbonyl groups, we designed an alternative route of synthesis involving an efficient coupling procedure without protection of the C-terminal carboxylic group. In peptide synthesis, the active ester method using the *N*-hydroxysuccinimide (ONSu) ester of N-protected amino acids has been successfully used to couple them with unprotected amino acids.[5,6] The ONSu ester, however, does not couple with the hydroxyl group of lactic acid, which is less nucleophilic than the amino group of amino acids. Another approach involves the use of an *N*-carboxyanhydride (NCA) with an unprotected amino acid as a starting material.[7-9] Such a reaction is more likely to succeed with lactic acid as the nucleophile. In fact, it has been shown that the NCAs react with benzyl alcohol to give the benzyl ester of the amino acids.[10-13] Thus, we employed the reaction of the NCAs with lactic acid. The NCAs were used as the Nps derivatives.[14,15] These compounds are more useful for stepwise synthesis than the unprotected NCAs since they do not form byproducts resulting from polymerization.[16]

As a model reaction, N^{α}-Nps-N^{ϵ}-Z-lysine NCA was treated with lactic acid benzyl ester in tetrahydrofuran at room temperature. The reaction was followed by thin layer chromatography using silica gel and chloroform–tetrahydrofuran (94:6) as an eluent. After 5 h, the TLC showed a main spot at R_f 0.7 and a faint spot at R_f 0.3. After isolation and purification by column chromatography, an oily material showing a single spot on TLC with R_f 0 was obtained in 85% yield. The IR absorption spectrum of the product showed three C=O stretching bands at 1750, 1725, and 1700 cm^{-1} which can be assigned as that of the Lys-Lac linkage, the C-terminal benzyl ester, and the side-chain urethane of lysine, respectively. These assignments were made on the basis of previously synthesized depsipeptide monomers in which the main-chain ester absorption generally appears near 1750 cm^{-1} and the C-terminal benzyl ester near 1725 cm^{-1}. Thus, the isolated material was identified as Nps-Lys(Z)-Lac-OBzl. The result of this model reaction clearly shows that this procedure provides a new method for depsipeptide synthesis with some advantages over the CDI-mediated coupling method owing to the rapid formation of the depsipeptide ester linkage without side reactions.

The synthetic route which was applied to the synthesis of the "monomers" used for preparation of polydepsipeptides containing polar side chains is shown in Scheme I. We attempted to prepare N-protected didepsipeptide free acids by the reaction of Nps-NCAs with unprotected lactic acid. Nps-Lys(Z)-NCA was treated with lactic acid, but no reaction occurred. This result is not surprising if one considers the low nucleophilicity of an α-hydroxy acid.

Scheme I



We therefore added an equivalent of pyridine to destroy the intramolecular hydrogen bonding of lactic acid and enhance the nucleophilicity of the hydroxyl group. The reaction of NCA with lactic acid proceeded rapidly in the presence of pyridine and the product after neutralization showed a single spot on TLC. It was identified as Nps-Lys(Z)-Lac-OH by IR and NMR spectra. Nps-Glu(OBzl)-Lac-OH and Nps-Glu(OMe)-Lac-OH were synthesized in high yield by the reaction of lactic acid in pyridine with Nps-NCAs of γ-benzyl glutamate and γ-methyl glutamate, respectively. These successful examples of depsipeptide synthesis using Nps-NCAs suggest that this new method may be widely applicable to other depsipeptide systems which we are now studying.

Elongation of the chain from the didepsipeptides described above to tridepsipeptides is also accomplished by using Nps-NCAs. This step of the synthesis is also quite similar to that of peptide synthesis which involves reaction of an Nps-NCA with an amino group at the N-terminal position of a peptide.[17] The Nps-didepsipeptide was treated with hydrochloric acid in dioxane to remove the Nps protecting group to give the didepsipeptide hydrochloride (step 2 in Scheme I). The resulting colorless oil was dissolved in tetrahydrofuran and treated with Nps-NCA in the presence of triethylamine (NEt$_3$, step 3 in Scheme I). The reaction was found to be complete in 1 h by TLC. The product was easily isolated and purified by column chromatography. The Nps-tridepsipeptide free acids thus obtained in high yield as a half-solid material showed a single spot on TLC.

During the final step of the monomer synthesis (step 4 in Scheme I), the Nps-tridepsipeptide free acids were converted to the active esters by a well-established method which involved a dicyclohexylcarbodiimide (DCCI) mediated reaction with pentachlorophenol.[18] The products, Nps-tridepsipeptide pentachlorophenol esters, were obtained as crystals and easily purified by recrystallization from tetrahydrofuran or ethanol. Table I lists the results of syntheses of these N-protected "monomers" together with their physical properties.

Two of these, Nps-Ala-Glu(OBzl)-Lac-OPcp and Nps-Ala-Lys(Z)-Lac-OPcp, were also prepared by another synthetic route, which is shown in Scheme II. This route involves esterification of Nps-didepsipeptide free acids with pentachlorophenol followed by chain elongation to give Nps-tridepsipeptide active esters. This synthesis, the "back-up" technique,[19] has an advantage that the oily N-protected didepsipeptide free acids could be converted

Table I
N-Protected Tridepsipeptide Pentachlorophenyl Esters

product	mp, °C	$[\alpha]_D$, deg (c 1.0% THF)	R_f I[a]	R_f II[b]	elemental anal.[c] % C	% H	% N
Nps-L-Ala-L-Glu(OBzl)-(*S*)-Lac-OPcp	92-93	-61.7	0.82	0.81	46.09	3.35	5.37
					46.14	3.42	5.42
Nps-L-Ala-L-Glu(OMe)-(*S*)-Lac-OPcp	159-160	-65.2	0.82	0.78	40.84	3.14	5.95
					40.70	3.23	5.87
Nps-L-Ala-L-Lys(Z)-(*S*)-Lac-OPcp	136-137	-57.4	0.68	0.66	46.59	3.79	6.79
					46.64	3.92	6.73
Nps-L-Glu(OMe)-L-Glu(OMe)-(*S*)-Lac-OPcp	142-143	-56.9	0.86	0.77	41.68	3.37	5.42
					41.59	3.47	5.38
Nps-L-Lys(Z)-L-Glu(OMe)-(*S*)-Lac-OPcp	175-176	-45.7	0.81	0.64	46.87	3.93	6.25
					46.75	3.97	6.24

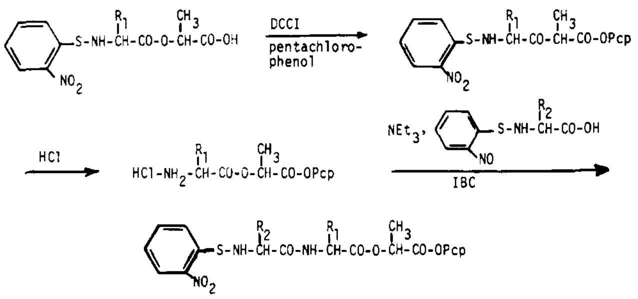
[a] Solvent system: chloroform-tetrahydrofuran (9:1). [b] Solvent system: chloroform-ethyl acetate (9:1). [c] Top row lists calculated values; bottom row lists found values.

Table II
Polydepsipeptides Obtained by Solution-State Polymerization

polymer	% yield	mp, °C	$[\alpha]_D$, deg (c 0.5% $CHCl_3$)	$[\eta]$,[a] dL/g	MW[b]
poly[L-Ala-L-Glu(OBzl)-(*S*)-Lac]	86	140-145	-20.8	0.20	28 000
poly[L-Ala-L-Glu(OMe)-(*S*)-Lac]	85	180-183	-17.4	0.41	60 000
poly[L-Ala-L-Lys(Z)-(*S*)-Lac]	79	100-105	-39.8	0.17	23 000
poly[L-Glu(OMe)-L-Glu(OMe)-(*S*)-Lac]	96	213-215	-16.3	0.55	82 000
poly[L-Lys(Z)-L-Glu(OMe)-(*S*)-Lac]	96	250-255	-18.7	0.85	129 000

[a] In dichloroacetic acid at 25 °C. [b] Calculated from a viscosity-molecular weight relationship.[21,22]

Scheme II



to highly crystalline C-activated derivatives which are easily purified by simple recrystallization. Thus, the Nps-didepsipeptides were treated with pentachlorophenol in the presence of dicyclohexylcarbodiimide to give the Nps-didepsipeptide pentachlorophenol esters, which were treated with hydrochloric acid in dioxane. The resulting didepsipeptide active ester hydrochlorides were obtained as fine white crystals and purified by recrystallization from ethanol. The didepsipeptide active esters were then treated with Nps-alanine in the presence of isobutyl chloroformate (IBC) to give the Nps-tridepsipeptide active esters. The physical properties of the N-protected "monomers" thus obtained by both routes shown in Schemes I and II were completely consistent.

Initial attempts to polymerize these "monomers" involved matrix-mediated thermal polymerization on Celite which has been developed in our laboratory and has been successfully used to obtain high molecular weight polydepsipeptides[2] as well as polypeptides.[20] Since only "monomers" having nonpolar side chains have been polymerized by this method thus far, it was of special interest to determine whether this method could be applied to "monomers" having polar side chains. In this study, the "monomers" were synthesized with residues of glutamic acid whose side chains were benzyl or methyl esters or lysine whose ϵ-amino groups were protected by the benzyloxycarbonyl group. These polar side chains were considered to be potentially unstable and could cause side reactions at the high temperatures generally used in the matrix-mediated thermal polymerization. The "monomers", TFA-H-Ala-Glu(OBzl)-Lac-OPcp and TFA-H-Ala-Lys(Z)-Lac-OPcp, were polymerized under the standard conditions of the matrix-mediated polymerization (on Celite at 100 °C for 2 days). The polymers obtained in ~60% yield had high viscosities ($[\eta]$ = 0.17–0.19 dL/g). However, analyses of these polymers by IR and NMR spectroscopy suggested that some side reactions occurred during thermal polymerization. These results will be discussed later in detail.

The "monomers" were then polymerized by a solution method. This method has been successfully employed to obtain many sequential polypeptides having not only nonpolar side chains but also polar side chains.[21] However, the molecular weights of the resulting polymers are rather low. In this study, the "monomers" were polymerized in *N,N*-dimethylacetamide. The polymerization reaction proceeded in solution to give the polydepsipeptide in a high yield. Results of the polymerization are listed in Table II. Viscosity of the polymers suggests that they are of very high molecular weight. For example, poly[Lys(Z)-Glu(OMe)-Lac] has an intrinsic viscosity, $[\eta]$, of 0.85 dL/g in dichloroacetic acid, which corresponds to a molecular weight of 130 000 calculated from a viscosity–molecular weight relationship.[21,22] It is also interesting that these depsipeptides having polar side chains exhibit higher solubility in solvents (chloroform, tetrahydrofuran, *N*,*N'*-dimethylformamide, dimethyl sulfoxide, halogenated alcohols, and organic acids) which are commonly used for conformational studies than polydepsipeptides containing alkyl side chains.

Conformational studies of these polydepsipeptides are now being undertaken and preliminary CD measurements indicate that all these polymers show stable α-helical conformations in tetrahydrofuran and trifluoroethanol. A detailed conformational study is reported in the paper following this one.

Discussion

The synthetic method using Nps-NCAs introduced in

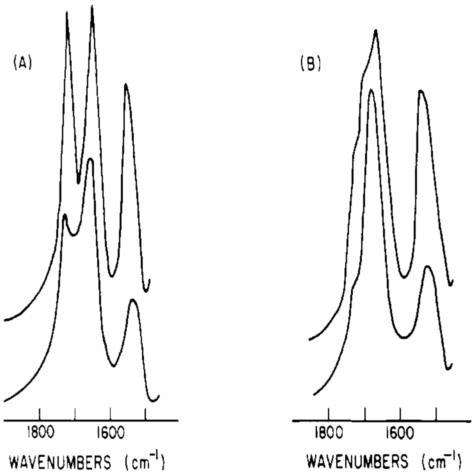


Figure 1. Infrared spectra of poly[Ala-Glu(OBzl)-Lac] (A) and poly[Ala-Lys(Z)-Lac] (B) obtained from solution-state polymerization (top spectra) and from thermal polymerization (bottom spectra). Obtained as KBr pellets. The ordinate is increasing absorbance.

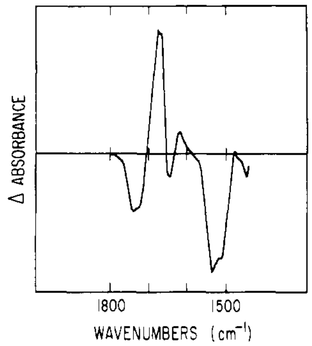


Figure 2. Difference IR spectrum of poly[Ala-Lys(Z)-Lac] obtained by subtracting the spectrum observed for the polymer obtained by solution polymerization from that obtained by thermal polymerization. The spectra were obtained in KBr pellets. The ordinate corresponds to the change in absorbance so that a positive change indicates an increase of absorption at a particular frequency.

this paper offers a new route for depsipeptide synthesis with some advantages over the known method using CDI coupling. While the CDI method requires a long reaction time (3 days or longer), the new method using Nps-NCAs takes only 5 h for completion of the coupling reaction. This rapid reaction of the Nps-NCAs can prevent undesirable side reactions and produce pure products in a high yield. Moreover, the new method can be used to prepare the depsipeptide free acid as well as the ester directly. This special feature of the new method has great importance for the synthesis of depsipeptides having polar side chains. This paper indicates some advantages of the syntheses using this route. The removal of the C-terminal protecting group is frequently unsuccessful for longer peptide chains. The Nps-NCA method could be used to prepare complicated longer depsipeptide fragments without C-terminal protection. Since the Nps-NCAs have been shown to produce peptides with full optical purity,[16] the depsipeptides obtained by this method are clearly also optically pure.

The "monomers" were polymerized by a thermally induced solid-state method as well as by condensation in solution initiated by addition of triethylamine. In the case of polymerization of the "monomers" with nonpolar side chains studied thus far, the polydepsipeptides obtained by the thermal polymerization on Celite have always been of higher molecular weights than those obtained by polymerizations in solution. However, in this paper, which involved the polymerization of "monomers" with polar side chains, the polymerizations in solution gave much better results. The resulting polydepsipeptides in solution retain the "monomer" sequences and exhibit high molecular weights. The polydepsipeptides containing a [Glu(OMe)]$_2$-Lac sequence had a very high molecular mass ((6–12.9) × 10^4 daltons) while the other polydepsipeptides containing [Glu(OBzl)]$_2$-Lac or Lys(Z)-Glu(OMe)-Lac sequences possessed lower molecular masses ((2.3–2.8) × 10^4 daltons). It is likely that the difference arises from the fact that we were able to purify the "monomers" having the former sequence by repeated recrystallizations from alcohol since they are very highly crystalline while the latter "monomers" were not as crystalline.

The polydepsipeptides obtained by thermal polymerization all had much lower intrinsic viscosity, $[\eta] = 0.15\text{–}0.17$ dL/g. We believe that these polymers also contain some incorrect structures resulting from side reactions during polymerization. Figure 1 illustrates the FT IR spectra in the ester and amide I and amide II regions of poly[Ala-Glu(OBzl)-Lac] and poly[Ala-Lys(Z)-Lac] obtained by both solid state thermal polymerization on Celite and solution polymerization. The polydepsipeptide poly[Ala-Glu(OBzl)-Lac] obtained in solution showed the bands of the ester, amide I, and amide II with the proper intensities expected for the two ester and two amide linkages. On the other hand, the same polymer obtained by the thermal method failed to show the relative intensities for these bands. The absorption of the C=O stretching of the ester and N–H deformation (amide II) are also anomalously weak as compared to absorption of the C=O stretching of the amide I. This suggests that some linkages (possibly the side-chain benzyl ester) and protons attached with amide nitrogen might be lost during polymerization.

The NMR spectrum of the polymer supports such conclusions. The aromatic and methylene protons of the benzyl group showed resonance peaks having areas 10% less than those of other protons.

The same differences in the IR bands were observed for the polydepsipeptide poly[Ala-Lys(Z)-Lac] when samples obtained by thermal polymerization on Celite were compared to samples obtained by solution polymerization. The correct assignment of differences in band strengths is somewhat unclear for this polymer since the polymer has one ester, one urethane, and two amide groups in each repeating unit. These bands are close enough to overlap with each other. We, therefore, took the infrared difference spectrum of these polymers by FT IR, which is shown in Figure 2. The difference spectrum clearly shows that the sample obtained by the thermal polymerization gives bands with reduced intensity at 1737 and 1715 cm^{-1} (overlapped ester and urethane C=O) and 1543 cm^{-1} (amide II) and a band with higher strength at 1687 cm^{-1} than those for the sample obtained by the polymerizations.

In conclusion, this study has shown that the polydepsipeptides with protected polar side chains are better prepared by the polymerizations in solution than by the thermal polymerization on Celite. However, these successful results obtained by the method of condensations in solution could not always be used for all depsipeptide systems. The polymerizations in solution may be affected by many factors which include the solubility of both the "monomer" and the resulting polymer and the growing depsipeptide chains. The method based on the thermal polymerization on Celite generally gives better results for many polypeptides and polydepsipeptides with nonpolar side chains. Further study will be necessary to define the scope and limitations for the polymerization of the "monomers". Ultimately we hope to be able to suggest optimum routes for the polymerization of specific sequence-controlled peptides and depsipeptides.

Experimental Section

***N*-(*o*-Nitrophenyl)sulfenyl α-Amino Acid *N*-Carboxyanhydrides (Nps-NCAs).** The N-substituted NCAs were prepared by reaction of the NCAs of L-alanine, γ-benzyl L-glutamate, γ-methyl L-glutamate, and ε-benzyloxycarbonyl-L-lysine with (*o*-nitrophenyl)sulfenyl chloride. The Nps-NCAs were highly crystalline compounds with the exception of γ-benzyl L-glutamate, which was obtained as an oil and showed physical properties identical with those reported by one of us (R.K.).[16]

α-Nps-ε-Benzyloxycarbonyl-L-lysyl-(*S*)-lactic Acid Benzyl Ester. The reactant Nps-L-Lys(Z)-NCA (2.1 g, 4.6 mmol) was dissolved in 20 mL of tetrahydrofuran, and (*S*)-lactic acid benzyl ester[1] (1.0 g, 5.5 mmol) was added to the solution. The solution was allowed to stand for 5 h at room temperature. The solution was then concentrated under reduced pressure to give a yellow oily residue. The oil was dissolved in 200 mL of ethyl acetate, and a small amount of undissolved material was removed by filtration. The filtrate was washed with 10% citric acid, water, 10% NaHCO$_3$, and water and dried over MgSO$_4$. The solvent was removed under reduced pressure to give an oil. An IR spectrum of this crude product indicated there was a small amount of unreacted Nps-NCA. To remove this contaminant, the oil was chromatographed over silica gel eluted with chloroform–tetrahydrofuran (9:1). A major yellow band was collected. The solvent was removed under reduced pressure, and the resulting oil was pumped under high vacuum for 24 h to give 1.9 g (85%) of the product, which showed a single spot on TLC at R_f 0.92 (ethyl acetate–hexane (1:1)) and 0.60 (chloroform–hexane (1:1)): $[\alpha]_D$ 33.4° (*c* 1.0% tetrahydrofuran); IR (neat) 1750, 1725, 1700, 1590, 1560 cm^{-1}; NMR (deuteriochloroform) δ 1.40 (d, 3 H), 1.55 (m, 6 H), 3.10 (m, 2 H), 4.30 (m, 1 H), 5.12 (s, 2 H), 5.20 (m, 1 H), 5.22 (s, 2 H), 7.38 (s, 10 H), 7.95 (m, 4 H).

α-Nps-ε-Benzyloxycarbonyl-L-lysyl-(*S*)-lactic Acid. The reactant (*S*)-lactic acid (4.8 g, 53.3 mmol) was dissolved in 100 mL of tetrahydrofuran, and dry pyridine (4.3 mL, 53.0 mmol) was added to the solution. To the solution was added Nps-L-Lys(Z)-NCA (15.0 g, 35.7 mmol) and the solution was allowed to stand for 5 h at room temperature. After the addition of Nps-NCA, many bubbles appeared, which suggested that the coupling reaction produced carbon dioxide. After the reaction, the solution was diluted with 200 mL of ethyl acetate and washed with 10% citric acid and water. The solution was extracted with aqueous NaHCO$_3$ solution, but little extract was obtained. The solution was then extracted with highly diluted NaOH solution. The extract was immediately acidified with concentrated citric acid aqueous solution. The aqueous solution was extracted with ethyl acetate. The solution was washed with water saturated with NaCl and dried over MgSO$_4$. The solvent was evaporated to give an oil product. The oil was chromatographed (silica gel column, chloroform–tetrahydrofuran (9:1) as an eluent) to give 11.2 g (79%) of the product, which moved as a single spot on TLC at R_f 0.27 (chloroform–methanol (9:1)) and 0.60 (chloroform–methanol (8:2)): $[\alpha]_D$ −29.3° (*c* 1.0% tetrahydrofuran); IR (neat) 1750, 1700, 1690, 1560 cm^{-1}; NMR (deuteriochloroform) δ 1.42 (d, 3 H), 1.80 (m, 6 H), 3.20 (m, 2 H), 3.60 (m, 1 H), 5.18 (s, m, 3 H), 7.32 (s, 5 H), 7.89 (m, 4 H).

α-Nps-γ-Benzyl-L-glutamyl-(*S*)-lactic Acid. This compound was prepared in 87% yield by reacting Nps-L-Glu(OBzl)-NCA with (*S*)-lactic acid in the presence of pyridine. R_f 0.20 (chloroform–methanol (9:1)) and 0.54 (chloroform–methanol (8:2)): $[\alpha]_D$ 36.9° (*c* 1.0% tetrahydrofuran); IR (neat) 1734, 1590, 1560 cm^{-1}; NMR (deuteriochloroform) δ 1.58 (d, 2 H), 2.30 (t, 2 H), 2.65 (d, 2 H), 3.68 (t, 1 H), 5.20 (s, m, 3 H), 7.40 (s, 5 H), 7.90 (m, 4 H).

α-Nps-γ-Methyl-L-glutamyl-(*S*)-lactic Acid. The reactant (*S*)-lactic acid (3.96 g, 44 mmol) was dissolved in 50 mL of tetrahydrofuran, and 7.0 mL of pyridine was added. To the solution was added Nps-Glu(OMe)-NCA (10 g, 29.3 mmol) and the solution was stirred for 4 h at room temperature. The solution was diluted with 200 mL of ethyl acetate, washed with 10% citric acid and water, and extracted with 10% NaHCO$_3$. The extract was washed with water and dried over MgSO$_4$. The solvent was evaporated under reduced pressure to give an oil. Hexane was added to the oil to give crystals. The product was recrystallized from warm ethyl acetate: yield 10.5 g (93%); mp 124–125 °C; R_f 0.13 (chloroform–methanol (9:1)) and 0.53 (chloroform–methanol (8:2)); $[\alpha]_D$ −64.5° (*c* 1.0% tetrahydrofuran); IR (KBr disk) 1755, 1736, 1665, 1590, 1560 cm^{-1}; NMR (deuteriochloroform) δ 1.55 (d, 3 H), 2.25 (t, 2 H), 2.60 (d, 2 H), 3.70 (s, 3 H), 3.80 (m, 1 H), 5.25 (m, 1 H), 7.90 (m, 4 H).

α-Nps-ε-Benzyloxycarbonyl-L-lysyl-γ-methyl-L-glutamyl-(*S*)-lactic Acid Pentachlorophenyl Ester. A Typical Example of N-Protected Monomers. The reactant Nps-L-Glu(OMe)-(*S*)-Lac-OH (7.7 g, 20 mmol) was dissolved in 40 mL of tetrahydrofuran, and 10 mL of 4 N hydrochloric acid in dioxane was added. The solvent was immediately removed under reduced pressure to give a yellow oil. The oil was extracted with diethyl ether until the yellow color disappeared. The oil was then dissolved in a small amount of THF and reprecipitated by addition of diethyl ether and hexane. The resulting colorless oil was dissolved in 100 mL of THF, and 3 mL of triethylamine was added. The resulting crystals of triethylammonium chloride were removed by filtration. To the filtrate was added with stirring Nps-L-Lys(Z)-NCA (9.2 g, 20 mmol). The solution was stirred for 1 h at room temperature, diluted with 300 mL of ethyl acetate, washed with 10% citric acid and water, and dried over MgSO$_4$. The solvent was evaporated under reduced pressure. To the resulting oil was added hexane to give 9.6 g (82%) of Nps-L-Lys(Z)-L-Glu-(OMe)-(*S*)-Lac-OH as a half-solid material: R_f 0.25 (chloroform–methanol (9:1)) and 0.61 (chloroform–methanol (8:2)); $[\alpha]_D$ 31.7° (*c* 1.0% tetrahydrofuran); IR (KBr disk) 1735, 1700, 1660, 1590, 1560 cm^{-1}.

The tridepsipeptide free acid (7 g, 10.8 mmol) was dissolved in 200 mL of tetrahydrofuran, and pentachlorophenol (3.4 g, 13 mmol) was added. The solution was cooled to −10 °C. Dicyclohexylcarbodiimide (2.7 g, 13 mmol) was added and the solution was stirred for 2 h at −10 °C and allowed to stand overnight in a refrigerator. The resulting crystals of dicyclohexylurea were removed by filtration, and the filtrate was diluted with 300 mL of ethyl acetate. The solution was rapidly washed with 10% citric acid, water, 5% NaHCO$_3$, and water and dried over MgSO$_4$. The solvent was evaporated under reduced pressure to give yellow crystals. The product was purified by recrystallization from hot methanol to give a pure Nps-L-Lys(Z)-L-Glu-(OMe)-(*S*)-Lac-OPcp.

All other N-protected tridepsipeptide OPcp esters were similarly prepared and purified by recrystallization. The results of the syntheses and the physical properties of the products are listed in Table I.

Nps-L-Alanyl-γ-benzyl-L-glutamyl-(*S*)-lactic Acid Pentachlorophenyl Ester. An Alternate Route to the N-Protected Monomers. The reactant Nps-L-Glu(OBzl)-(*S*)-Lac-OH (11 g, 23.8 mmol) was dissolved in 100 mL of tetrahydrofuran, and pentachlorophenyl (8.2 g, 31 mmol) was added to the solution. The solution was cooled to −10 °C, and dicyclohexylcarbodiimide (5.9 g, 28.6 mmol) was added with stirring. The solution was stirred for 3 h at −10 °C and then at 0 °C. The reaction was monitored by TLC. After 5 h, the starting free acid, Nps-didepsipeptide, nearly disappeared at R_f 0.0 (chloroform). Only a strong yellow spot at R_f 0.50 was shown. The resulting crystals were removed by filtration, and the filtrate was diluted with 200 mL of ethyl acetate, washed with 10% citric acid, water, 10% NaHCO$_3$, and water, and dried over MgSO$_4$. The solvent was removed under reduced pressure, and the resulting oil was dissolved in a small amount of ethyl acetate. The crystals of dicyclohexylurea were removed by filtration, and the filtrate was concentrated under reduced pressure. Pumping the resulting oil gave 13.5 g (80%) of Nps-L-Glu(OBzl)-(*S*)-Lac-OPcp as an amorphous powder: IR (KBr disk) 1794, 1740, 1590, 1560, 1510 cm^{-1}; NMR (deuteriochloroform) δ 1.78 (d, 3 H), 2.31 (t, 2 H), 2.62 (d, 2 H), 3.74 (m, 1 H), 6.00 (s, 2 H), 5.60 (q, 1 H), 7.38 (s, 5 H), 7.90 (m, 4 H).

The depsipeptide derivative, Nps-L-Glu(OBzl)-(*S*)-Lac-OPcp (13 g, 18.3 mmol) was dissolved in 100 mL of tetrahydrofuran, and 10 mL of 4 N HCl in dioxane was added. The solvent was removed under pressure to give yellow crystals. The crystals were triturated with ether, collected on a glass filter, and washed with ether until the yellow color disappeared. Then the crystals were dissolved in warm ethanol and recrystallized by addition of ether to give quantitatively colorless crystals of HCl·H-L-Glu(OBzl)-(*S*)-Lac-OPcp: mp 167–168 °C (dec); IR (KBr disk) 1782, 1765, 1725 cm^{-1}, NMR (trifluoroacetic acid) δ 1.88 (d, 2 H), 2.59 (t, 2 H), 2.96 (d, 2 H), 4.62 (m, 1 H), 5.11 (s, 2 H), 5.70 (q, 1 H), 7.25 (s, 5 H), 7.70 (br, 3 H).

The reactant Nps-L-alanine (2.4 g, 15 mmol) was dissolved in 50 mL of tetrahydrofuran, and 2.1 mL of triethylamine was added. The solution was cooled to −15 °C. Isobutyl chloroformate (IBC, 15 mmol) was added. After 5 min, a solution of HCl·H-L-Glu-(OBzl)-(*S*)-Lac-OPcp (5.9 g, 10 mmol) in 50 mL of *N*,*N*-dimethylformamide was added to the solution, and 2.1 mL of triethylamine in 10 mL of tetrahydrofuran was added dropwise over 15 min. The solution was stirred for 1 h at 0 °C. The solution was then diluted in 300 mL of ethyl acetate, washed with 10% citric acid, water, 10% NaHCO$_3$, and water, and dried over MgSO$_4$. The solvent was evaporated under reduced pressure to give an oily residue. The oil was triturated in ether–hexane to give a yellow powder. The product was recrystallized from ethyl acetate to give a pure Nps-L-Ala-L-Glu(OBzl)-(*S*)-Lac-OPcp which has the same physical properties as those of the sample prepared with the alternate route (see Table I): IR (KBr disk) 1790, 1740, 1655, 1590, 1560, 1540 (sh), 1510 cm^{-1}; NMR (deuteriochloroform) δ 1.50 (d, 3 H), 1.85 (d, 3 H), 2.55 (m, 4 H), 3.78 (m, 1 H), 4.86 (m, 1 H), 5.28 (s, 2 H), 5.70 (q, 1 H), 7.54 (s, 5 H), 7.90 (m, 4 H).

Polymerization of the Monomers. Matrix-Mediated Polymerization on Celite. Nps-L-Ala-L-Glu(OBzl)-(*S*)-Lac-OPcp (2.06 g, 2.5 mmol) was dissolved in 10 mL of trifluoroacetic acid (TFA). After 30 min, ether and hexane were added to the solution. The resulting oily product was repeatedly extracted with a mixture of ether–hexane to give a colorless oil. The oil was then dissolved in a small amount of tetrahydrofuran and reprecipitated by addition of ether. The oil was triturated in ether to give pure TFA·H-L-Ala-L-Glu-(OBzl)-(*S*)-Lac-OPcp as a powder. The active ester TFA salt (1 g, 1.27 mmol) was dissolved in 20 mL of chloroform, and 1.5 g of Celite was added. The solvent was evaporated to give a white solid. This procedure was repeated an additional two times. The solid was dried in vacuo for 20 h at 25 °C in a sublimator. The polymerization vessel was heated at 110 °C under a high vacuum (0.2 mmHg) for 2 days. After cooling, the solid material was extracted with trifluoroethanol. The extract was concentrated under reduced pressure. Addition of ether gave a pale yellow polymer, 0.26 g (56%).

Poly(L-Ala-L-Lys(Z)-(*S*)-Lac) was prepared by the same method in 59% yield.

Solution-State Polymerization, Poly[L-Lys(Z)-Glu-(OMe)-(*S*)-Lac]. Nps-L-Lys(Z)-L-Glu(OMe)-(*S*)-Lac-OPcp (9.6 g, 6.7 mmol) was dissolved in 20 mL of tetrahydrofuran, and 3.5 mL of 4 N HCl in dioxane was added. The solvent was evaporated under reduced pressure to give a yellow solid. Then the product was collected in a glass filter, washed with ether, and recrystallized from tetrahydrofuran to give pure HCl·H-L-Lys(Z)-L-Glu-(OMe)-(*S*)-Lac-OPcp (4.9 g, 94%). The active ester HCl salt (4.6 g, 5.9 mmol) was dissolved in 9.2 mL of *N*,*N*-dimethylacetamide. With vigorous stirring, 1 mL of triethylamine was added. The solution was stirred for 2 days at room temperature. The solution was then diluted with 500 mL of water to precipitate the polymer. The precipitate was dissolved in 300 mL of chloroform, and the resulting solution was washed with water and dried over MgSO$_4$. The solvent was evaporated to give an oil. Addition of ether gave a white powder. The product was collected on a filter, washed with methanol and ether, and dried in vacuo; yield 2.7 g (96%).

Measurements. NMR spectra were recorded with a Varian T-60 spectrometer and optical rotations were measured on a Perkin-Elmer 141 polarimeter. IR spectra for characterization of the intermediates were obtained with a Perkin-Elmer 180 spectrophotometer and FT IR spectra were obtained with a Nicolet 7199 FT IR spectrophotometer.

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References and Notes

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