Synthesis and Conformational Analysis of Optically Active Poly(*β*-peptides)

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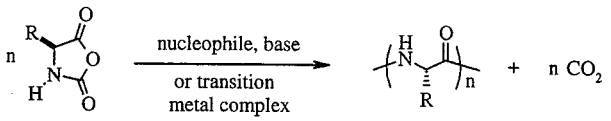
ABSTRACT: Optically active poly(*β*-peptides) with proteinogenic side chains were synthesized via the polymerization of *β*-amino acid-*N*-carboxyanhydrides (*β*-NCAs) initiated using either NaO*t*Bu or a nickel amido amidate complex. Although most of these low molecular weight poly(*β*-peptides) have poor solubility in common organic solvents, those that were soluble were found to adopt stable chiral conformations in solution. Poly(N_{ξ}-carbobenzyloxy-*β*-L-homolysine) (**2f**) was observed to adopt a helical conformation in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), which could be disrupted by addition of methanesulfonic acid (MSA), a strong denaturing agent. The side-chain deprotected polymers, poly(*β*-L-homolysine) (**3**) and poly(*β*-L-homoglutamate) (**4**), were found to display pH-dependent conformation transitions in aqueous solution.

Introduction

The synthesis and characterization of peptides consisting of *β*-amino acids, so-called *β*-peptides, is a research field that has received considerable interest in recent years.[1−4] *β*-Amino acids are conformationally more flexible when compared to α-amino acids because of their additional α-methylene unit. It was once thought that the introduction of these additional freely rotating C−C *σ*-bonds would result in a decreased ability for *β*-peptides to adopt ordered solution conformations relative to the α-peptides. However, Seebach has demonstrated[2a] that *β*-peptides containing as few as six monomer repeats can surprisingly adopt stable secondary structures in solution, while α-peptide chains typically require more than 15 residues to do so. It was later found by Seebach and Gellman that *β*-peptides in solution can fold into all of the regular chain conformations that are observed in proteins (i.e., helix, sheet, and turn).[1,2] They have conducted detailed studies on how residue structure controls *β*-peptide conformation in solution and found that helix sense, helix size (placement of intramolecular hydrogen bonding that stabilizes the helix), and overall conformation can be controlled through adjustment of side-chain substituents at the α- and/or *β*-carbons on the *β*-peptide backbone.

Methods for facile preparation of high molecular weight poly(*β*-peptides) (i.e., >100 residues) are scarce and limited to specific cases.[5,6] The formation of *β*-peptides through a polymerization process offers a potential advantage over the tedious stepwise solid-state methods commonly used to prepare these materials. Through polymerization, many *β*-amino acid residues can be coupled in a single procedure, which is readily scaled up. The resulting poly(*β*-peptides) also have considerable potential for biomedical applications including drug delivery and as therapeutics.[1h,2f,4] The only poly(*β*-peptides) that have attracted much attention in the past few decades are the poly(α-alkyl-*β*-aspartates) mainly due to the availability of aspartic acid,[6] the only naturally occurring proteinogenic *β*-amino acid. To expand the range of these materials, we sought to develop a general methodology for synthesis of optically active poly(*β*-peptides) containing the side chains of natural α-amino acids.

The chemical synthesis of high molecular weight poly(α-peptides) is most readily accomplished by the ring-opening polymerization of α-amino acid-*N*-carboxyanhydride (α-NCA) monomers (eq 1).[7] However, NCA



α-NCA (1)

ring-opening polymerization has not been well explored for the synthesis of poly(*β*-peptides), primarily since no general method for efficient synthesis of optically pure *β*-amino acid-*N*-carboxyanhydrides (*β*-NCAs) from amino acids has been developed. Recently, we have successfully synthesized optically pure *β*-NCAs from the cyclization of N_{β}-*t*-Boc- or N_{β}-*t*-Cbz-*β*-amino acids (Scheme 1).[8] Here we report the polymerization of these *β*-NCAs as well as studies on the physical properties and solution conformations of the resulting optically pure polymers.

Results and Discussion

Polymerizations of *β*-NCAs (Table 1) were first attempted in THF solution using the initiator NaO*t*Bu, which is generally useful in α-NCA polymerizations for synthesis of high molecular weight poly(α-peptides). The yields of **2a**−**d,** polymers bearing small hydrophobic side chains, were generally very high (entries 1−4). However, the molecular weights of these polymers were low due to precipitation of the chains during synthesis, leading to chain transfer and termination reactions. **1a** polymerized most rapidly, mostly likely since it has the smallest side chain of the *β*-NCAs studied and is least sterically hindered. Polymerization of **1a** was complete within 12 h (entry 1), while polymerizations of **2b**−**d** required either additional time or elevated temperatures to reach completion (entries 2−4). Larger NCA substituents are believed to hinder access of the propagating chain ends to the monomer anhydride groups, thus slowing the polymerizations.[7] Consequently, polymerizations of *β*-NCAs with sterically demanding side chains (**1e** and **1f**) were found to be very slow (entries

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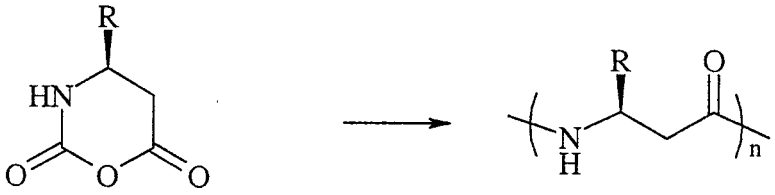
Scheme 1. Polymerization of Optically Active *β*-Amino acid-*N*-carboxyanhydrides (*β*-NCAs)



1a-f **2a-f**

Entry	R	2
a	$-CH_3$	Poly(β-L-homoalanine)
b	$-CH(CH_3)_2$	Poly(β-L-homovaline)
c	$-CH_2CH(CH_3)_2$	Poly(β-L-homoleucine)
d	$-CH_2C_6H_5$	Poly(β-L-homophenylalanine)
e	$-(CH_2)_2C(O)OCH_2C_6H_5$	Poly(N_δ-benzyl-β-L-homoglutamate)
f	$-(CH_2)_4NHC(O)OCH_2C_6H_5$	Poly(N_ξ-carbobenzyloxy-β-L-homolysine)

Table 1. Polymerization of *β*-NCAs

entry	initiator	substrate	M/I	solvent	time (h)	temp (°C)	product	yield (%)	M_n[b]	DP
1	NaOtBu	**1a**	50	THF	12	20	**2a**	96	970	11
2	NaOtBu	**1b**	50	THF	20	20	**2b**	95	1310	12
3	NaOtBu	**1c**	50	THF	15	60	**2c**	97	1680	13
4	NaOtBu	**1d**	50	THF	20	60	**2d**	95	1370	9
5	NaOtBu	**1e**	50	THF	72	60	**2e**	88	2380	11
6	NaOtBu	**1e**	50	DMF	90	60	**2e**	78	3440	16
7	NaOtBu	**1e**	100	DMF	90	60	**2e**	65	3740	17
8	NaOtBu	**1f**	50	THF	72	65	**2f**	85	2470	10
9	NaOtBu	**1f**	50	DMF	96	65	**2f**	74	3940	14
10	depeNiAA[a]	**1e**	100	CH_2Cl_2	120	35	**2e**	73	3210	15
11	depeNiAA	**1e**	100	THF	72	60	**2e**	72	2150	10
12	depeNiAA	**1e**	100	DMF	90	60	**2e**	61	4330[c]	20
13	depeNiAA	**1f**	100	THF	72	60	**2f**	78	2210	8
14	depeNiAA	**1f**	100	DMF	72	60	**2f**	85	3530[d]	13

[a] See text. [b] Measured using MALDI-TOF MS. DP = average degree of polymerization. [c] $[\eta] = 0.362$ in DCA. [d] $[\eta] = 0.289$ in DCA.

5−8). Precipitation also occurred when polymerizations of **1e** and **1f** were carried out in THF solution; as a result, only low molecular weight chains were obtained (**2e** and **2f**). When polymerization of **1e** or **1f** was carried out in DMF solution at elevated temperature, the polymers **2e** and **2f** were obtained with slightly greater chain lengths (entries 6 and 9). A nickel complex that efficiently polymerizes α-NCAs, (bis-1,2-((($CH_3CH_2)_2$P)-CH_2CH_2)Ni(NHCH(CH(CH_3)$_2$)C(O)NC(CH_3)$_3$) (depeNiAA),[9] was also used for *β*-NCA polymerizations (Table 1). Similar results were observed in that polymers prepared in DMF solution generally had higher molecular weights than those synthesized in THF (entries 11−14). Other α-NCA polymerization initiators, such as $Co(PMe_3)_4$ and $Pt(PMe_3)_4$,[10] gave results similar to those found with depeNiAA. Under all conditions studied, polymerizations of *β*-NCAs **1a**−**f** gave polymers with low molecular weights likely due to precipitation of the polymers from the reaction mixtures. This appears to be a general phenomenon, since to the best of our knowledge, no high molecular weight poly(*β*-peptides) have been synthesized via the polymerization of *β*-NCAs.[7a]

Oligomers **2a**−**c** have similar solubilities in common organic solvents (Table 2). They are insoluble in THF and methylene chloride but partially soluble in DMF and hexafluoro-2-propanol (HFIP). All the *β*-peptide oligomers could be readily dissolved in trifluoroacetic acid (TFA) except **2d**, which is only partially soluble in this solvent. Oligo(*β*-L-homophenylalanine) (**2d**) displays

Table 2. Solubility of Oligo(*β*-peptides)

sample	THF	CH_2Cl_2	DMF	HFIP	TFA
2a	I[a]	I	PS[b]	PS	S[c]
2b	I	I	PS	PS	S
2c	I	I	PS	PS	S
2d	I	I	PS	PS	PS
2e	I	I	S	S	S
2f	I	I	S	S	S

[a] I = insoluble (<0.2 mg/mL). [b] PS = partially soluble (0.2−2 mg/mL). [c] S = soluble (>2 mg/mL).

the poorest solubility, being insoluble in DMF and only slightly soluble in HFIP. Conversely, oligo(*δ*-benzyl-*β*-L-homoglutamate) (**2e**) is complete soluble in DMF and HFIP but is insoluble in THF and methylene chloride. Oligo(*δ*-carbobenzyloxy-*β*-L-homolysine) (**2f**) possesses solubility characteristics similar to **2e**. Generally, poly(*β*-peptides) have poorer solubilities in organic solvents when compared to their α-analogues. For instance, poly(*γ*-benzyl-α-L-glutamate) with molecular weight up to 100 kDa is soluble in THF,[11] while the *β*-analogue **2e** is insoluble in this solvent even at much lower molecular weight (Table 2). The poor solubility of poly(*β*-peptides) is likely due to either polymer−polymer interchain hydrogen bonding or crystallization of conformationally ordered chains. The existence of interchain H bonding would suggest that the chains adopt some degree of either interchain *β*-sheet like or disordered conformations in solution, as opposed to purely intrachain H-bonded helical conformations.

To study this issue in more detail, conformational analysis of these polymers was performed using circular

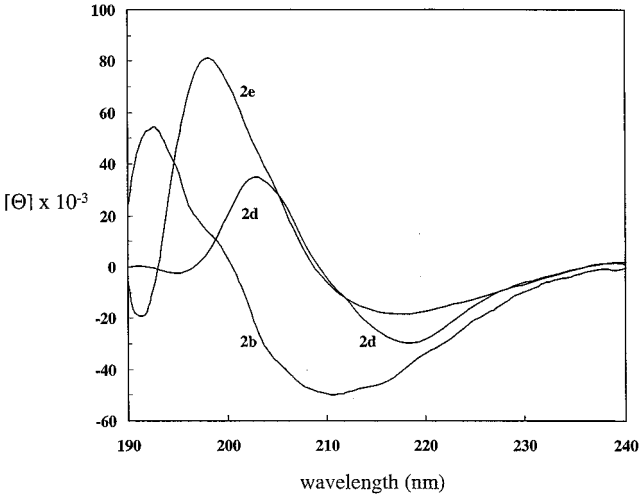


Figure 1. CD spectra of oligo(β-HVal) **2b**, oligo(β-HPhe) **2d**, and oligo(β-HGlu(Bn)) **2e** in HFIP. Polymer concentrations = 0.5 mg/mL. M_n of **2b**, **2d**, and **2e** are 1310, 1370, and 3940, respectively.

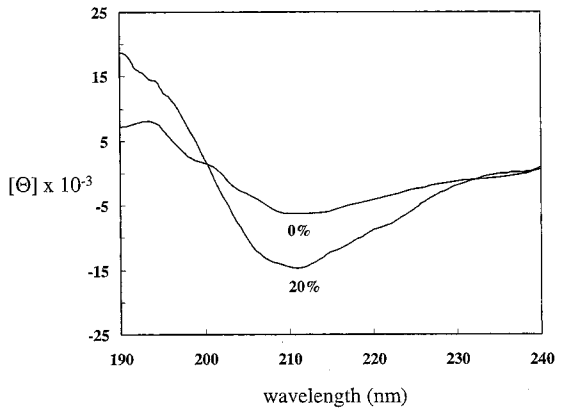


Figure 2. CD spectra of oligo(β-HLys(Cbz)) **2f** in HFIP mixed with different amounts of H_2O. Numbers refer to % H_2O by volume in solution. Polymer concentrations = 0.5 mg/mL. M_n of **2f** = 3580.

dichroism (CD) spectroscopy. The CD spectrum of **2b** in HFIP possessed a minimum at 211 nm and a maximum at 192 nm with molar ellipticities of -4.93×10^4 and 5.43×10^4 deg cm^2/mol, respectively (Figure 1). These spectral features are very similar to those described by Seebach for the heptamer of L-β-homolysine, which was reported to adopt a 3_1 helical conformation.[2d] The oligomer **2d** gave a CD spectrum similar to that of **2b**, except both the minimum (-2.98×10^4 deg cm^2/mol at 218 nm) and the maximum (3.49×10^4 deg cm^2/mol at 202 nm) bands were shifted to higher wavelengths due to contributions from the phenyl side chains.[12] The CD spectrum of **2e** in HFIP gave a weak minimum at 217 nm (-1.83×10^4 deg cm^2) but a strong maximum at 198 nm (8.11×10^4 deg cm^2). The CD pattern of **2e** is very similar to that of (β-HAsp-β-HSer-β-HGlu)$_2$, which also adopts a 3_1 helical conformation as reported by Seebach.[2f]

When **2f** was analyzed in trifluoroethanol (TFE) solution, a CD spectrum indicative of a random coil conformation was observed.[13] However, the CD spectrum of **2f** in HFIP shows a weak minimum at 211 nm and a weak maximum at 193 nm with molar ellipticities of -6.7×10^3 and 8.1×10^3 deg cm^2/mol, respectively (Figure 2). This CD pattern remained unchanged as the concentration of **2f** was varied from 0.5 to 2 mg/mL,

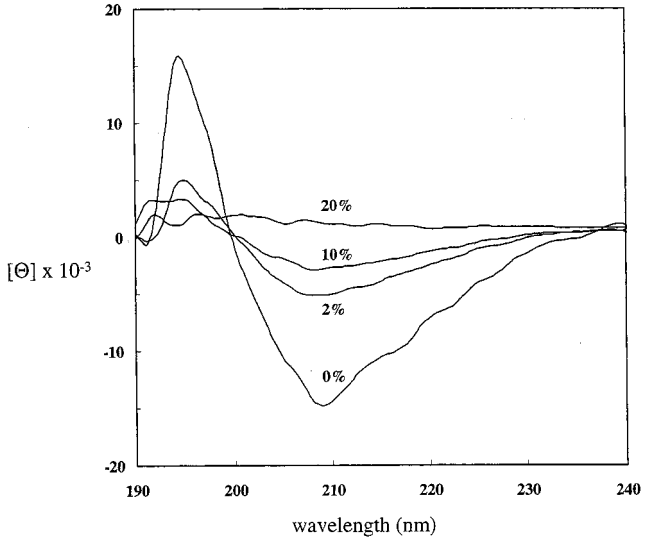
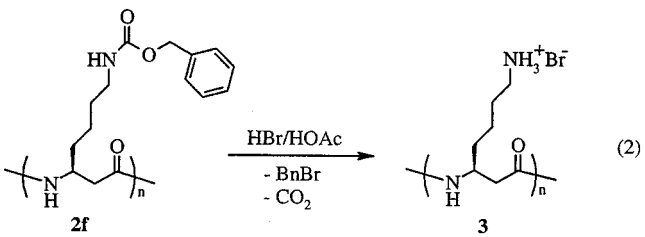


Figure 3. CD spectra of oligo(β-HLys(Cbz)) **2f** in a mixture of 75% HFIP and 25% H_2O. Numbers refer to the % of MSA by volume in each solution. Polymer concentrations = 0.5 mg/mL. M_n of **2f** = 3580.

showing that the oligomer is not aggregated in HFIP. The addition of water to the HFIP solution of **2f** (20% v/v H_2O in HFIP) was found to significantly affect the conformation of the polymer as the intensities of both CD bands were found to increase dramatically (Figure 2). The molar ellipticities of both bands remained constant as the polymer concentration was varied from 0.2 to 1 mg/mL, indicating no polymer aggregation in the solvent mixture. This solvent mixture is believed to be more acidic when compared to that of neat HFIP.[14] Thus, intramolecular H-bonding should be strengthened in the solvent mixture due to desolvation of the hydrophobic portions of the polymer, stabilizing the ordered conformation.[5d] When the H_2O content was increased to 30% v/v, the polypeptide began to precipitate from solution.

Methanesulfonic acid (MSA) has been found to be a good UV-transparent denaturant for CD analysis of polypeptides.[15] Polypeptides are usually in disordered conformations in neat MSA because the solvent disrupts the hydrogen bonding that stabilizes ordered chain conformations. Since **2f** in 25% v/v H_2O in HFIP gives an intense CD spectrum, MSA was added to this solution to see whether the presumed helical conformation could be denatured. The intensities of both minimum and maximum CD bands were found to decrease when as little as 2% MSA was added to the **2f** solution, indicating partial disruption of the ordered conformation. When the percentage of MSA in the solvent mixture was increased to 20%, the disruption of helix to coil was complete (Figure 3).

The deprotected poly(β-homolysine) (**3**) was prepared by hydrolysis of **2f** with HBr (eq 2). Since the side chains



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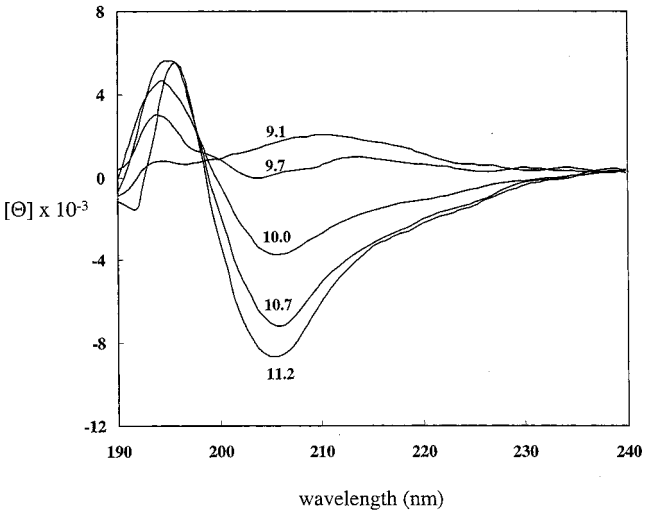


Figure 4. CD spectra of oligo(β-HLys) **3** in aqueous solution. Numbers in spectra refer to solution pH. Polymer concentrations = 0.5 mg/mL. M_n of **3** = 1760.

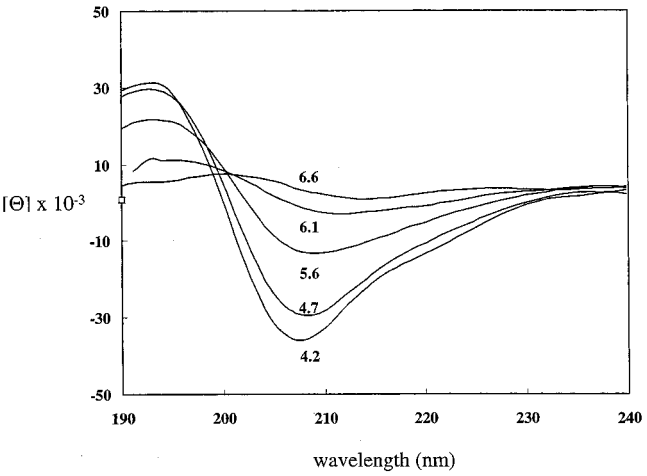
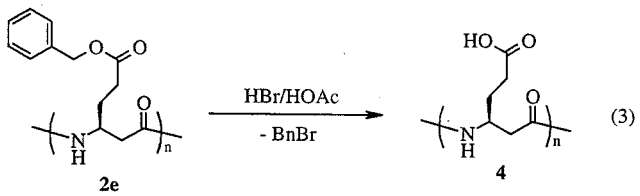


Figure 5. CD spectra of oligo(β-HGlu) **4** in aqueous solution. Numbers in spectra refer to Solution pH. Polymer concentrations = 0.5 mg/mL. M_n of **4** = 1550.

of **3** are positively charged at low pH, the conformation of **3** under these conditions is disordered due to electrostatic repulsion of neighboring charges.[16] As revealed by CD, **3** possessed a random conformation in aqueous solutions when the pH was less than 9.7. When pH was increased, a transition from a coil to a helical conformation occurred at ca. pH 9.7−10. The intensities of both minimum and maximum bands increased as pH was raised from 10 to 11.2, indicating complete transition from coil to helix. The CD bands corresponding to the helical conformation disappeared when the pH was readjusted to 9.5, verifying the reversibility of this transition. A similar pH-dependent helix−coil transition in aqueous solutions of poly(α-lysine) is well-known.[16]

In aqueous solutions of deprotected oligo(β-homoglutamate) (**4**) (eq 3), whose side chains are negatively



(3)

charged at high pH, a random coil-to-helix transition was also observed when the solution was acidified (Figure 5). A sharp transition was observed at pH values between 5.6 and 6.1. The most intense CD bands were observed at pH 4.2, while adjustment of solution pH to lower values resulted in polymer precipitation and decreased CD signal intensity.

Experimental Section

General. Tetrahydrofuran, hexane, dichloromethane, dimethylformamide, and diethyl ether were dried by passage through alumina under nitrogen prior to use.[17] Chemicals were purchased from commercial supplies and used without purification. MALDI(TOF) mass spectra were collected using a Thermo BioAnalysis DYNAMO mass spectrometer running in positive ion mode with samples prepared by mixing solutions of analyte in TFA with solutions of 6-aza-2-thiothymine in TFA and allowing the mixture to air-dry. Viscosity measurements were made in dichloroacetic acid (DCA) solution using an Ubbelohde type capillary viscometer at 25 ± 0.1 °C. NMR spectra were recorded on a Bruker AVANCE 200 MHz spectrometer. Circular dichroism measurements were carried out on an Olis rapid scanning monochromator running in conventional scanning mode at room temperature. The path length of the quartz cell was 1.0 mm, and the concentration of polypeptide was 0.2−1.0 mg/mL. Optical rotations of poly(β-peptides) were measured in either dichloroacetic acid (DCA) or trifluoroacetic acid (TFA) on a Jasco model P1020 polarimeter using a 1 mL volume cell (1 dm length) at a concentration of 10 mg/mL. Infrared spectra were recorded on a Perkin-Elmer RX1 FTIR spectrophotometer calibrated using polystyrene film. Deionized water (18 MΩ·cm) was obtained by passing in-house deionized water through a Barnstead E-pure purification system. Preparation of β-amino acid-*N*−carboxyanhydrides (β-NCAs) for the synthesis of poly(β-peptides) was performed as previously described.[8] (Bis-1,2-((CH$_3$CH$_2$)$_2$P)CH$_2$-CH$_2$)Ni(NHCH(CH(CH$_3$)$_2$)C(O)NC(CH$_3$)$_3$) (depeNiAA) was prepared as previously described.[9] Co(PMe$_3$)$_4$ and Pt(PMe$_3$)$_4$ were synthesized as previously described.[10]

General Procedure of the polymerization of β-NCAs. In the drybox, the β-NCA (0.1 mmol) was dissolved in THF (or DMF) (0.5 mL) and placed in a 25 mL reaction tube which could be sealed with a Teflon stopcock. An aliquot of initiator (100 μL of a 0.05 M solution in THF) was then added via syringe to the flask. A stirbar was added, and the flask was sealed, removed from the drybox, and stirred at room temperature for 3 days. Polymer was precipitated from the THF (or DMF) suspension by addition of the reaction mixture to methanol. The solid polymer was washed with methanol twice and dried under high vacuum.

Poly(β-homo-L-alanine) (2a). Polymerization of β-HAla NCA (**1a**) was carried out in THF using NaO*t*Bu. Polymer yield was 96%. Molecular weight analysis using MALDI(TOF)-MS: M_n = 970 g/mol. FT-IR (KBr): 1650 cm^{-1} (νCO, amide I, s), 1552 cm^{-1} (νCO, amide II, s). ^{1}H NMR (TFA-d): δ 4.56 (br m, 1H, −(NHC*H*(CH$_3$)CH$_2$CO)$_n$−), 3.08 (br m, 2H, −(NHCH-(CH$_3$)C*H*$_2$CO)$_n$−), 1.54 (br dd, 3H, −(NHCH(C*H*$_3$)CH$_2$CO)$_n$−). [α]$_D^{25}$ (DCA) −17.0.

Poly(β-homo-L-valine) (2b). Polymerization of β-HVal NCA (**1b**) was carried out in THF using NaO*t*Bu. Polymer yield was 95%. Molecular weight analysis using MALDI(TOF)-MS: M_n = 1310 g/mol. FT-IR (KBr): 1651 cm^{-1} (νCO, amide I, s), 1550 cm^{-1} (νCO, amide II, s). ^{1}H NMR (TFA-d): δ 4.49 (br m, 1H, −(NHC*H*(CH(CH$_3$)$_2$)CH$_2$CO)$_n$−), 3.18 (br m, 2H, −(NHCH(CH(CH$_3$)$_2$)C*H*$_2$CO)$_n$−), 2.11 (br m, 1H, −(NHCH-(C*H*(CH$_3$)$_2$)CH$_2$CO)$_n$−), 1.30 (br dd, 6H, −(NHCH(CH(C*H*$_3$)$_2$)-CH$_2$CO)$_n$−). [α]$_D$25 (TFA) + 10.1.

Poly(β-homo-L-leucine) (2c). Polymerization of β-HLeu NCA (**1c**) was carried out in THF using NaO*t*Bu. Polymer yield was 97%. Molecular weight analysis using MALDI(TOF)-MS: M_n = 1680 g/mol. FT-IR (KBr): 1651 cm^{-1} (νCO, amide I, s), 1550 cm^{-1} (νCO, amide II, s). ^{1}H NMR (TFA-d): δ 4.51 (br m, 1H, −(NHC*H*(CH$_2$CH(CH$_3$)$_2$)CH$_2$CO)$_n$−), 3.16 (br m, 2H,

−(NHCH(CH$_2$CH(CH$_3$)$_2$)C*H*$_2$CO)$_n$−), 2.18−1.72 (br dd, 3H, −(NHCH(C*H*$_2$C*H*(CH$_3$)$_2$)CH$_2$CO)$_n$−), 1.23 (br, 6H, −(NHCH-(CH$_2$CH(C*H*$_3$)$_2$)CH$_2$CO)$_n$−). $[\alpha]_D^{25}$ (TFA) −74.1.

Poly(β-homo-L-phenylalanine) (2d). Polymerization of β-HPhe NCA (**1d**) was carried out in THF using NaO*t*Bu. Polymer yield was 95%. Molecular weight analysis using MALDI(TOF)-MS: M_n = 1370 g/mol. FT-IR (KBr): 1652 cm^{-1} (ν*C*O, amide I, s), 1547 cm^{-1} (ν*C*O, amide II, s). ^{1}H NMR (TFA-d): δ 7.45 (br, 5H, −(NHCH(CH$_2$C$_6$*H*$_5$)CH$_2$CO)$_n$−), 4.59 (br, 1H, −(NHC*H*(CH$_2$C$_6$H$_5$)CH$_2$CO)$_n$−), 3.37−2.82 (br, 4H, −(NHCH(C*H*$_2$C$_6$H$_5$)C*H*$_2$CO)$_n$−). $[\alpha]_D^{25}$ (DCA) −19.0.

Poly(β-N_δ-benzyl-homo-L-glutamate) (2e). Polymerizations of β-HGlu(Bn) NCA (**1f**) were carried out either in THF or DMF using either NaO*t*Bu or a transition metal complex. Polymer yields were in a range from 61% to 88%. Molecular weight analysis using MALDI(TOF)-MS: M_n ranged from 2150 to 4330 g/mol. FT-IR (CH$_2$Cl$_2$): 1736 cm^{-1} (ν*C*O, ester, s), 1652 cm^{-1} (ν*C*O, amide I, s), 1547 cm^{-1} (ν*C*O, amide II, s). ^{1}H NMR (TFA-d): δ 8.08 (br s, 5H, −(NHCH(CH$_2$CH$_2$COOCH$_2$C$_6$*H*$_5$)CH$_2$-CO)$_n$−), 5.95 (br s, 2H, −(NHCH(CH$_2$CH$_2$COOC*H*$_2$C$_6$H$_5$)CH$_2$-CO)$_n$−), 5.05 (br m, 1H, −(NHC*H*(CH$_2$CH$_2$COOCH$_2$C$_6$H$_5$)CH$_2$-CO)$_n$−), 3.31 (br m, 4H, −(NHCH(CH$_2$C*H*$_2$COOCH$_2$C$_6$H$_5$)C*H*$_2$-CO)$_n$−), 2.75 (br m, 2H, −(NHCH(C*H*$_2$CH$_2$COOCH$_2$C$_6$H$_5$)CH$_2$-CO)$_n$−). $[\alpha]_D^{25}$ (DCA) −8.1, (TFA) −6.9.

Poly(β-N_ξ-carbobenzyloxy-homo-L-lysine) (2f). Polymerizations of β-HLys(Cbz) NCA (**1e**) were carried out either in THF or DMF using either NaO*t*Bu or depeNiAA. Polymer yields ranged from 74% to 85%. Molecular weight analysis using MALDI(TOF)-MS: M_n ranged from 2210 to 3940 g/mol. FT-IR (CH$_2$Cl$_2$): 1720 cm^{-1} (ν*C*O, carbamate, s), 1652 cm^{-1} (ν*C*O, amide I, s), 1547 cm^{-1} (ν*C*O, amide II, s). ^{1}H NMR (TFA-d): δ 7.64 (br s, 5H, −(NHCH(CH$_2$CH$_2$CH$_2$CH$_2$NHCOOCH$_2$-C$_6$*H*$_5$)CH$_2$CO)$_n$−), 5.56 (br s, 2H, −(NHCH(CH$_2$CH$_2$CH$_2$CH$_2$-NHCOOC*H*$_2$C$_6$H$_5$)CH$_2$CO)$_n$−), 4.54 (br m, 1H, −(NHC*H*(CH$_2$-CH$_2$CH$_2$CH$_2$NHCOOCH$_2$C$_6$H$_5$)CH$_2$CO)$_n$−), 3.63 (br m, 2H, −(NHCH(CH$_2$CH$_2$CH$_2$C*H*$_2$NHCOOCH$_2$C$_6$H$_5$)CH$_2$CO)$_n$−), 2.96 (br m, 2H, −(NHCH(CH$_2$CH$_2$CH$_2$CH$_2$NHCOOCH$_2$C$_6$H$_5$)C*H*$_2$-CO)$_n$−), 1.87−1.48 (br m, 6H, −(NHCH(C*H*$_2$C*H*$_2$C*H*$_2$CH$_2$-NHCOOCH$_2$C$_6$H$_5$)CH$_2$CO)$_n$−). $[\alpha]_D^{25}$ (DCA) −3.7.

Viscosity Measurements. Polymer solution viscosities were measured by comparing the time (t) required for a specific volume of polymer solution to flow through a capillary tube compared to the time (t_0) for pure solvent. Specific viscosity (η_{sp}) and intrinsic viscosity ([η]) are given by $\eta_{sp} = (t - t_0)/t_0$ and $[\eta] = [(\ln(t/t_0)/C]_{c=0}$. [$\eta$] was obtained by plotting η_{sp}/C against C (C = concentration of polymer solution in g/dL) according to the equation $\eta_{sp}/C = [\eta] + k'C$.

Either **2e** or **2f** (21.4 mg) was dissolved in DCA to give 13 mL of solution. The solution was then maintained in an Ubbelohde type capillary viscometer for 30 min at 25 ± 0.1 °C. The time (t) was then measured three times at this temperature, and the average of the data was calculated.

The η_{sp} of **2e** (entry 12, Table 1) and **2f** (entry 14, Table 1) were 0.175 and 0.153, respectively. The [η] of **2e** (entry 12, Table 1) and **2f** (entry 14, Table 1) were 0.362 and 0.289, respectively. The specific and intrinsic viscosities of both **2e** and **2f** indicate that they are low molecular weight oligomers.

Poly(β-homo-L-lysine) (3). To a TFA (5 mL) solution of **2f** (0.55 g, 2 mmol) in an ice bath was added 5 equiv of 33% HBr in acetic acid (w/w) with stirring. The mixture was warmed to room temperature and stirred for 1 h. The product was precipitated by addition of ether and then dried. The crude polymer was dissolved in a minimum amount of water/methanol solvent mixture (v/v = 1/2) and then precipitated using ether. The polymer was dried and then redissolved in water and dialyzed against water for 2 days. The aqueous solution was freeze-dried, and a white fluffy solid was obtained (0.25 g, 1.8 mmol, 92%). Molecular weight analysis using MALDI(TOF)-MS: 1760 g/mol. FT-IR (KBr): 1650 cm^{-1} (ν*C*O, amide I, s), 1550 cm^{-1} (ν*C*O, amide II, s). ^{1}H NMR (D$_2$O): δ 4.04 (br m, 1H, −(NHC*H*(CH$_2$CH$_2$CH$_2$CH$_2$NH$_3^+$)CH$_2$CO)$_n$−), 2.94 (br m, 2H−(NHCH(CH$_2$CH$_2$CH$_2$CH$_2$NH$_3^+$)C*H*$_2$CO)$_n$−), 2.33 (br m, 2H, −(NHCH(CH$_2$CH$_2$CH$_2$C*H*$_2$NH$_3^+$)CH$_2$CO)$_n$−), 1.58−1.05 (br m, 6H −(NHCH(C*H*$_2$C*H*$_2$C*H*$_2$CH$_2$NH$_3^+$)CH$_2$-CO)$_n$−).

Poly(β-homo-L-glutamate) (4). To a TFA solution (2 mL) of **2e** (1 mmol) in an ice bath was added 4 equiv of 33% HBr in acetic acid (v/v) with stirring. The mixture was warmed to room temperature and stirred for an additional 30 min. The product was precipitated by addition of ether. After ether was removed by centrifugation, the polymer was resuspended in a ether/methanol solvent mixture (v/v = 10/1) several times until a white powder was obtained. The polymer was collected and dried under vacuum (0.25 g, 1.75 mmol, 86%). Molecular weight analysis using MALDI(TOF)-MS: 1550 g/mol. FT-IR (KBr): 1650 cm^{-1} (ν*C*O, amide I, s), 1548 cm^{-1} (ν*C*O, amide II, s). ^{1}H NMR (10% NaOD/D$_2$O): δ 4.10 (br m, 1H, −(NHC*H*-(CH$_2$CH$_2$COONa)CH$_2$CO)$_n$−), 2.23 (br m, 4H, −(NHCH-(CH$_2$C*H*$_2$COONa)C*H*$_2$CO)$_n$−), 1.68 (br m, 2H, −(NHCH(C*H*$_2$-CH$_2$COONa)CH$_2$CO)$_n$−).

Conclusions

Optically active poly(β-peptides) **2a**−**f**, bearing side chains of natural occurring amino acids, were synthesized via the polymerization of β-NCAs. These polymerizations were initiated using either NaO*t*Bu or a transition metal complex. The molecular weights of **2a**−**f** were generally low due to the precipitation of these polymers from the polymerization solution. Some of these low molecular weight poly(β-peptides) adopt stable helical conformations in solution although most generally have poor solubility in common organic solvents. The oligomer **2f** was observed to adopt a helical conformation in HFIP which could be disrupted by addition of a strong denaturing agent such as methanesulfonic acid. The side-chain deprotected polymers, **3** and **4**, were found to display pH-dependent helix−coil conformation transitions in aqueous solution, similar to their α-analogues. Our results demonstrate that polymerization of β-NCAs can be used to rapidly prepare oligo-β-peptides that display characteristics similar to β-peptides prepared using stepwise procedures.

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