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Tyrosine-based poly(*m*-phenyleneethynylene-*p*-phenyleneethynylene)s. Helix folding and responsiveness to a base

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ABSTRACT

The Sonogashira–Hagihara polymerization of 3′,5′-diiodo-*N*-α-*tert*-butoxycarbonyl-L-tyrosine methyl ester (**1**) and 3′,5′-diiodo-*N*-α-*tert*-butoxycarbonyl-*O*-methyl-L-tyrosine methyl ester (**2**) with *para*-diethynylbenzene (**3**) was carried out to obtain optically active poly(*m*-phenyleneethynylene-*p*-phenyleneethynylene)s [poly(**1**) and poly(**2**)] with M_n's ranging from 9900 to 15,000 in 80–87% yields. Poly (**1**) exhibited intense CD signals in DMSO and THF, but did not in CH_2Cl_2, indicating that it took a predominantly one-handed helical conformation in the former two solvents. On the other hand, there was no evidence for poly(**2**) to take a helical structure in these solvents. Poly(**1**) turned the CD sign at 390 nm from plus to minus in DMSO/H_2O = 9/1 (v/v) by the addition of NaOH. Alkaline hydrolysis of ester moieties of poly(**1**) and poly(**2**) gave the corresponding polymers having carboxy groups [poly(**1a**) and poly(**2a**)]. Poly(**1a**) and poly(**2a**) increased the CD intensity by the addition of NaOH.

1. Introduction

A precisely ordered stereostructure of biopolymers such as peptide and DNA is a key importance for them to exhibit their biological functions and properties. Helix is the most common and fundamental secondary structure of synthetic polymers as well as biopolymers. The study on helical conformation in nonbiological polymers possibly enhances understanding of the mechanism for helix formation in biomacromolecules. Synthetic helical polymers are applicable to molecular recognition (separation, catalysis, enantioselective sensors), circularly polarized photo- and electroluminescent materials, and liquid crystalline materials [1]. Poly (phenyleneethynylene)s have attracted much attention owing to the features such as nonlinear optics, asymmetric electrodes, light polarization, photonic switching, chiral separation, and supramolecular organization [2]. Poly(*m*-phenyleneethynylene)s having polar side groups tend to fold into a helical structure in polar solvents due to the bended linkage and amphiphilic character, i.e., hydrophobic phenyleneethynylene backbones and hydrophilic side chains interacting with polar media. Moore and coworkers have reported that oligo(*m*-phenyleneethynylene)s with hydrophilic oligo(ethylene glycol) chains adopt a helical conformation thermodynamically driven by solvophobic effects [3]. Inouye and coworkers have synthesized poly(*m*-pyridinyleneethynylene)s with hydrophilic oligo(ethylene glycol) chains, and induced a helical conformation by protonation in protic media [4]. Poly(*m*-phenyleneethynylene-*p*-phenyleneethynylene)s are polymers consisting of conjugated *m*-phenyleneethynylene and *p*-phenyleneethynylene linkages alternatingly. Tew and coworkers have demonstrated that amphiphilic poly(*m*-phenyleneethynylene-*p*-phenyleneethynylene)s exhibit spectroscopic changes due to folding of the backbone into a helical conformation [5]. Schanze and coworkers have synthesized poly(*m*-phenyleneethynylene-*p*-phenyleneethynylene)s featuring sulfonate and carboxylate side groups that take a helical structure in water [6]. Zhu and coworkers have reported that poly(*m*-phenyleneethynylene-*p*-phenyleneethynylene)s containing pyridine units undergo conformation transition from an extended coil structure to a helical structure driven by solvents, and stabilized by metal ions [7]. All of these polymers require polar media to take helical forms because the driving force of helix folding is solvation of hydrophilic exterior (polar side groups) with polar solvents. A helical form is favorable for shielding the hydrophobic interior (phenyleneethynylene main chain) from polar media. On the other hand, we have recently found new examples of poly(*m*-phenyleneethynylene-*p*-phenyleneethynylene)s that form helices based on a mechanism different from that of poly(phenyleneethynylene) derivatives reported so far. Namely, hydroxyphenylglycine-based poly(*m*-phenyleneethynyl

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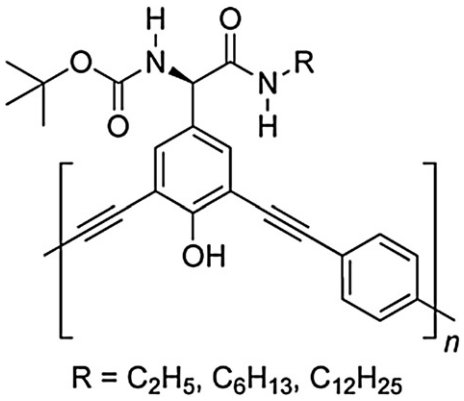


Chart 1. Hydroxyphenylglycine-based poly(*m*-phenyleneethynylene-*p*-phenyleneethynylene)s.

ene-*p*-phenyleneethynylene)s having hydrophilic phenolic hyd roxy group at the phenylene main chain and hydrophobic alkyl groups at the side chain (Chart 1) form helices in low polar organic solvents such as $CHCl_3$ and CH_2Cl_2 [8]. Addition of water decreases the helicity. It is considered that the key importance of this helix formation is amphiphilicity (hydrophobic exterior and hydrophilic interior), which is the reverse of poly(phenyleneethynylene) derivatives reported so far. Regulated intramolecular hydrogen bonding between the amide groups at the side chains also seems to be effective to stabilize the helical structure.

In the present article, we report the synthesis of tyrosine-based novel poly(*m*-phenyleneethynylene-*p*-phenyleneethynyl ene)s (Scheme 1), and elucidation of the effect of protection of the phenolic hydroxy and carboxy groups on the secondary structures, i.e., effect of amphiphilic balance between the interior and exterior of the polymers. We also report the effects of NaOH addition and solvent on the secondary structures.

2. Experimental part

2.1. Measurements

^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a JEOL EX-400 spectrometer. IR spectra were measured on a JASCO FTIR-4100 spectrophotometer. The number- and weight-average molecular weights (M_n and M_w) of polymers were determined by gel permeation chromatography (GPC) on a JASCO Gulliver system (PU-980, CO-965, RI-930, and UV-1570) equipped with polystyrene gel columns (Shodex columns K804, K805, and J806), using THF or DMF as an eluent calibrated by polystyrene standards at 40 °C. Melting points (mp) were measured on a Yanaco micro melting point apparatus. Elemental analysis was performed at the Microanalytical Center of Kyoto University. Specific rotations ($[\alpha]_D$) were measured on a JASCO DIP-1000 digital polarimeter. CD and UV–vis spectra were recorded using a quartz cell (path length: 1 cm) on a JASCO J-820 spectropolarimeter.

2.2. Materials

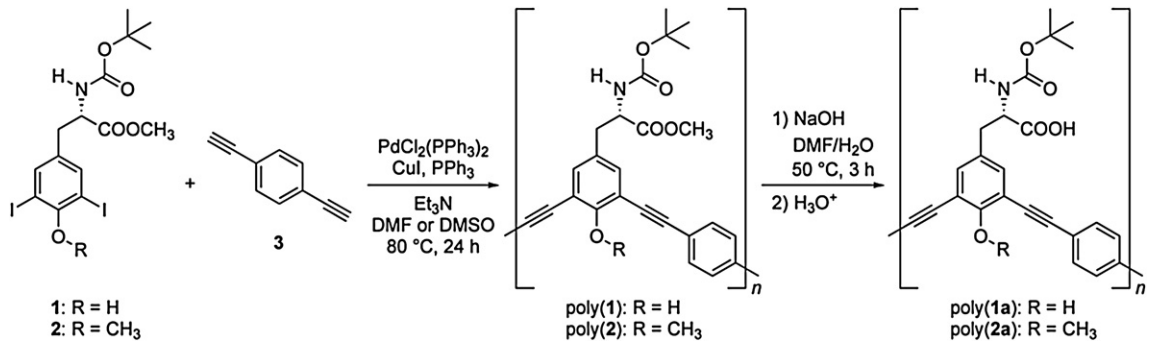
DMF, DMSO, and Et_3N used for polymerization were distilled over CaH_2 prior to use. All other reagents were commercially obtained, and used as received without purification.

2.3. Monomer synthesis

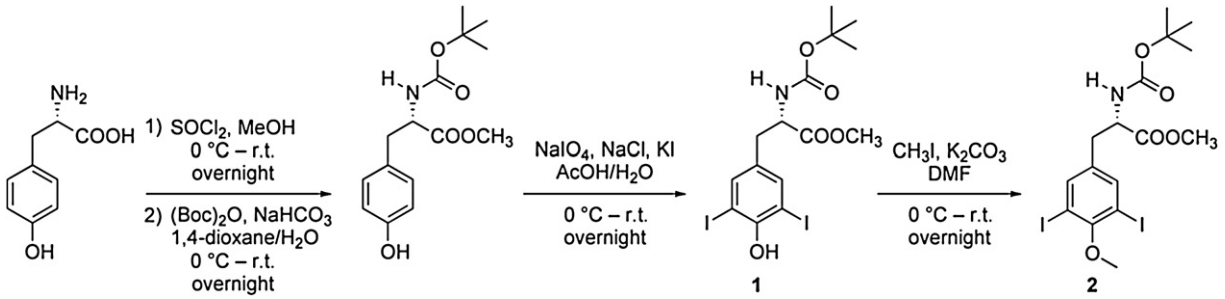
2.3.1. 3′,5′-Diiodo-N-α-tert-butoxycarbonyl-L-tyrosine methyl ester (**1**)

L-Tyrosine (10.9 g, 60.1 mmol) was suspended in MeOH (200 mL), and thionyl chloride (8.00 mL, 110 mmol) was added to the suspension dropwise at 0 °C. After the resulting mixture was stirred at room temperature overnight, the solvent was removed by rotary evaporation, and the residue was washed twice with ether to yield L-tyrosine methyl ester as a white solid (13.9 g, 60.0 mmol, 99%). A solution of di-*tert*-butyl dicarbonate (15.7 g, 71.5 mmol) in 1,4-dioxane (75 mL) was added to a mixture of L-tyrosine methyl ester (13.9 g, 60.0 mmol) and $NaHCO_3$ (7.55 g, 90.0 mmol) in H_2O (75 mL). The mixture was stirred at 0 °C for 1 h and then at room temperature overnight. After the mixture was acidified with 2 M HCl to approximately pH 2, it was extracted with three portions of AcOEt (100 mL). The combined organic layer was washed with H_2O (100 mL) and brine (100 mL), and then dried over $MgSO_4$. The solvent was removed by rotary evaporation to yield *N*-α-*tert*-butoxycarbonyl-L-tyrosine methyl ester as a white solid (16.8 g, 56.8 mmol, 94.6%).

N-α-*tert*-butoxycarbonyl-L-tyrosine methyl ester (5.31 g 18.0 mmol), NaCl (4.20 g, 72.0 mmol) and $NaIO_4$ (3.85 g, 18.0 mmol) were dissolved in AcOH/H_2O [9/1 (v/v), 60 mL]. The reaction mixture was stirred for 15 min. Then, KI (8.96 g, 54.0 mmol) was added at 0 °C, and the resulting mixture was stirred at room temperature overnight. After H_2O (200 mL) was added, the solution was extracted with $CHCl_3$. The organic layer was washed first with 1 M aqueous $Na_2S_2O_3 \cdot 5H_2O$ and saturated aq. NaCl, dried over anhydrous $MgSO_4$, and concentrated on a rotary evaporator. The residue was purified by silica gel column chromatography eluted with *n*-hexane/AcOEt [1/1 (v/v)] to obtain **1** as white powder in 77% yield (7.58 g, 13.8 mmol). Mp 126–127 °C, $[\alpha]_D = +18°$ ($c = 0.1$ g/dL, $CHCl_3$, room temperature). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.45$ [s, 9H, $(CH_3)_3$], 2.91–3.00 (m, 2H, ArCH_2), 3.74 (s, 3H, $COOCH_3$), 4.49 (s, 1H, NH*CH*COO), 5.03 (s, 1H, OH), 5.73 (s, 1H, CONH), 7.44 (s, 2H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 28.3, 36.3, 52.4, 54.3, 80.2, 82.1, 132.2, 140.0, 152.7, 155.6, 171.9$. IR (KBr): 3362, 2979, 1758, 1729, 1686, 1524, 1458, 1405, 1367, 1254,



Scheme 1. Sonogashira–Hagihara coupling polymerization of monomers **1** and **2** with **3** and alkaline hydrolysis of ester moieties of the obtained polymers.



Scheme 2. Synthesis of monomers **1** and **2**.

1165, 1019, 930, 850, 761, 647 cm^{-1}. Anal. Calcd for $C_{15}H_{19}I_2NO_5$: C, 32.93; H, 3.50; N, 2.56. Found: C, 32.94; H, 3.39; N, 2.57.

2.3.2. 3′,5′-Diiodo-*N*-α-*tert*-butoxycarbonyl-*O*-methyl-L-tyrosine methyl ester (**2**)

A solution of 3′,5′-diiodo-*N*-α-*tert*-butoxycarbonyl-L-tyrosine methyl ester (5.47 g, 10.0 mmol) in DMF (50 mL) was cooled using an ice bath. To the solution, freshly ground K_2CO_3 (1.53 g, 11.2 mmol), and then a cooled solution of iodomethane (0.70 mL, 1.59 g, 11.2 mmol) in DMF (20 mL) were added dropwise at 0 °C. The mixture was stirred at room temperature overnight. It was poured into ice water, and extracted with ethyl acetate. The organic layers were washed with water, and saturated aq. NaCl, dried over anhydrous $MgSO_4$, and concentrated on a rotary evaporator. The residue was purified by silica gel column chromatography eluted with *n*-hexane/AcOEt [4/1 (v/v)] to obtain **2** as white powder in 66% yield (3.7 g, 6.6 mmol). Mp 74–75 °C, $[\alpha]_D = +22°$ (c = 0.1 g/dL, $CHCl_3$, room temperature). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.45 [s, 9H, $(CH_3)_3$], 2.91–3.04 (m, 2H, CH_2Ar), 3.75–3.83 (m, 6H, CH_3O, $COOCH_3$), 4.52 (s, 1H, NHC*H*COO), 5.06 (s, 1H, CONH), 7.53 (s, 2H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.3, 36.4, 52.4, 54.2, 60.6, 80.1, 90.3, 136.1, 140.5, 154.8, 157.8, 171.7. IR (KBr): 3345, 2983, 2849, 1738, 1694, 1538, 1459, 1416, 1321, 1174, 1057, 911, 868, 727, 646 cm^{-1}. Anal. Calcd for $C_{16}H_{21}I_2NO_5$: C, 34.25; H, 3.77; N, 2.50. Found: C, 34.10; H, 3.65; N, 2.58.

2.4. Polymerization

All the polymerizations were carried out in a glass tube equipped with a three-way stopcock under nitrogen. A typical experimental procedure for polymerization of **1** with *para*-diethynylbenzene (**3**) is given below.

A solution of **1** (547 mg, 1.00 mmol), **3** (126 mg, 1.00 mmol), $PdCl_2(PPh_3)_2$ (35 mg, 50 μmol), CuI (4.7 mg, 25 μmol), PPh_3 (26.2 mg, 100 μmol), and Et_3N (2.00 mL, 14.3 mmol) in DMF (3 mL) was stirred at 80 °C for 24 h. After that, the resulting mixture was poured into MeOH/acetone [4/1 (v/v), 300 mL] to precipitate a polymer. It was separated by filtration using a membrane filter (ADVANTEC H100A047A) and dried under reduced pressure.

2.5. Alkaline hydrolysis of poly(**1**) and poly(**2**) [synthesis of poly(**1a**) and poly(**2a**)]

The ester groups of poly(**1**) and poly(**2**) were hydrolyzed under basic conditions. A typical experimental procedure for the hydrolysis of poly(**1**) to poly(**1a**) is given below.

Aqueous sodium hydroxide (10%, 20 mL) was added to a solution of poly(**1**) (250 mg, 0.62 mmol) in DMF (20 mL) dropwise at 0 °C, and then the resulting mixture was stirred at 50 °C for 3 h. The reaction mixture was poured into 2 M HCl (300 mL) to precipitate a polymer. It was separated by filtration using a membrane filter (ADVANTEC A010A047A) and dried under reduced pressure.

2.6. Spectroscopic data of the polymers

Poly(**1**): ^1H NMR (400 MHz, $CDCl_3$): δ = 1.43 (s, 9H, $(CH_3)_3$), 2.94–3.14 (m, 2H, $ArCH_2$), 3.74 (s, 3H, $COOCH_3$), 4.62 (s, 1H, NHC*H*CO), 5.10 (s, 1H, NHCOO), 7.26–7.71 (br, 6H, Ar). IR (KBr): 3427, 2975, 2207, 1714, 1600, 1408, 1365, 1278, 1166, 1013, 913, 840, 750, 625 cm^{-1}. Poly(**2**): ^1H NMR (400 MHz, $CDCl_3$): δ = 1.46 [s, 9H, $(CH_3)_3$], 2.94–3.11 (m, 2H, $ArCH_2$), 3.77–4.16 (m, 6H, OCH_3, $COOCH_3$), 4.57 (s, 1H, NHC*H*CO), 5.09 (s, 1H, NHCOO), 7.28–7.54 (br, 6H, Ar). IR (KBr): 3338, 2972, 2204, 1743, 1714, 1598, 1416, 1365, 1242, 1161, 1057, 1003, 836, 745, 634 cm^{-1}. Poly(**1a**): ^1H NMR (400 MHz, DMSO): δ = 1.28 [s, 9H, $(CH_3)_3$], 2.74–2.93 (m, 2H, $ArCH_2$), 4.18 (s, 1H, NHC*H*CO), 5.14 (s, 1H, NHCOO), 7.18–7.61 (br, 6H, Ar), 12.69 (s, 1H, COOH). IR (KBr): 3437, 2982, 2204, 1704, 1612, 1415, 1385, 1272, 1146, 983, 903, 839, 737, 645 cm^{-1}. Poly(**2a**): ^1H NMR (400 MHz, DMSO): δ = 1.89 [s, 9H, $(CH_3)_3$], 2.79–3.13 (m, 2H, $ArCH_2$), 3.74–3.96 (m, 3H, OCH_3), 4.83 (s, 1H, NHC*H*CO), 4.99 (s, 1H, NHCOO), 8.17–8.33 (br, 6H, Ar), 12.01 (s, 1H, COOH). IR (KBr): 3318, 2991, 2212, 1751, 1704, 1577, 1406, 1331, 1210, 1122, 1009, 957, 831, 771, 629 cm^{-1}.

3. Results and discussion

3.1. Monomer synthesis and polymerization

Novel diiodo compounds **1** and **2** were synthesized from L-tyrosine by the route illustrated in Scheme 2. First, *N*-α-*tert*-butoxycarbonyl-L-tyrosine methyl ester was synthesized by methyl esterification of L-tyrosine, followed by protection of amino group with *tert*-butoxycarbonyl (Boc) group. Then it was iodinated with potassium iodide, sodium periodate, and sodium chloride in acetic acid/water = 9/1 (v/v) to afford compound **1**. The hydroxy group of **1** was transformed into methyl ether with iodomethane in the presence of K_2CO_3 to obtain 3′,5′-diiodo-*N*-α-*tert*-butoxycarbonyl-*O*-methyl-L-tyrosine methyl ester. Both **1** and **2** were obtained as white powders and characterized by ^1H, ^{13}C NMR, and IR spectroscopies besides elemental analysis.

The polymers were synthesized by the Sonogashira–Hagihara polycondensation of **1** or **2** with **3** in Et_3N/DMF or Et_3N/DMSO at 80 °C for 24 h [9]. The corresponding polymers [poly(**1**) and poly(**2**)]

Table 1
Polycondensation of **1** and **2** with **3**[a].

Run	Monomer	Solvent	Yield[b] (%)	M_n[c]	M_w/M_n[c]
1	**1** + **3**	DMF	87	10,600	1.53
2	**1** + **3**	DMSO	84	9900	2.26
3	**2** + **3**	DMF	83	15,000	1.44
4	**2** + **3**	DMSO	80	13,200	1.72

[a] Conditions: $[\mathbf{1}]_0 = [\mathbf{2}]_0 = [\mathbf{3}]_0$ = 0.2 M, $[PdCl_2(PPh_3)_2]$ = 0.01 M, [CuI] = 0.005 M, $[PPh_3]$ = 0.02 M, Et_3N/solvent = 2/3 (v/v), 80 °C, 24 h.
[b] MeOH/acetone = 4/1 (v/v)-insoluble part.
[c] Determined by GPC eluted with THF calibrated by polystyrene standards.

with M_n's ranging from 9900 to 15,000 were obtained in 80–87% yields as listed in Table 1. The structures of the polymers were characterized spectroscopically. The ^{1}H NMR and IR spectra of the polymers exhibited signals reasonably assignable to the structures illustrated in Scheme 1. The polymers were soluble in CH_2Cl_2, $CHCl_3$, THF, DMF, and DMSO, while insoluble in *n*-hexane and MeOH.

3.2. Secondary structure of poly(**1**) and poly(**2**)

We measured the CD and UV–vis spectra of poly(**1**) in CH_2Cl_2, THF, and DMSO at room temperature. As shown in Fig. 1, poly(**1**) exhibited a very large positive CD signal around 390 nm in THF and DMSO, while no signal in CH_2Cl_2. In every case, poly(**1**) exhibited a UV–vis absorption peak around 350 nm, and the relative intensity agreed with that of the CD signals. The CD signals and UV–vis absorption peaks of poly(**1**) definitely come from the conjugated phenyleneethynylene backbone because the λ_{max}'s of the monomers locate at shorter wavelength regions (**1**: 285 and **3**: 276 nm) than those of the polymers. The similar CD and UV–vis spectroscopic patterns are also reported regarding a poly(*m*-phenyleneethynylene-*p*-phenyleneethynylene) featuring optically active carboxylate side groups [6b]. Thus, it is concluded that poly(**1**) takes a helical structure with predominantly one-handed sense in THF and DMSO [2]. Hence it is clear that the conformation is susceptible to solvent in a manner similar to poly(phenyleneethynylene)s [10]. We measured the ^{1}H NMR spectra of poly(**1**) in CD_2Cl_2 and DMSO-d_6 to find no apparent difference between them. Judging from the same λ_{max} of poly(**1**) in the solvents, it is considered that the conjugated length does not depend on the solvents. Consequently, it seems that poly(**1**) does not adopt a random conformation but a helical one in CH_2Cl_2 as well, wherein the ratio of right- and left-handed helices is 1:1, resulting in no CD signal.

Fig. 2 depicts the CD and UV–vis spectra of poly(**1**) measured in DMSO/H_2O with various compositions at room temperature. When the DMSO:H_2O proportion was 5:5, the [θ] of poly(**1**) became 63% of that in DMSO, while the ε_{max} remained at 93%. When the main

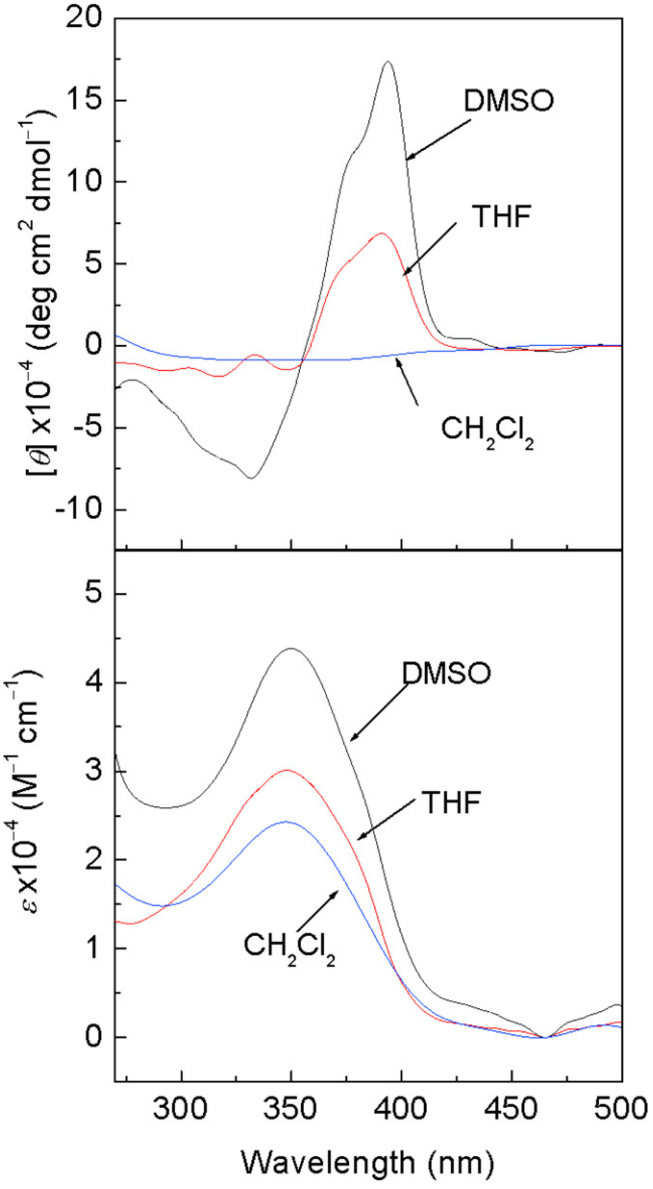


Fig. 1. CD and UV–vis spectra of poly(**1**) measured in CH_2Cl_2, THF, and DMSO ($c = 4.5 \times 10^{-5}$ mol L^{-1}) at room temperature. Sample: run 1 in Table 1.

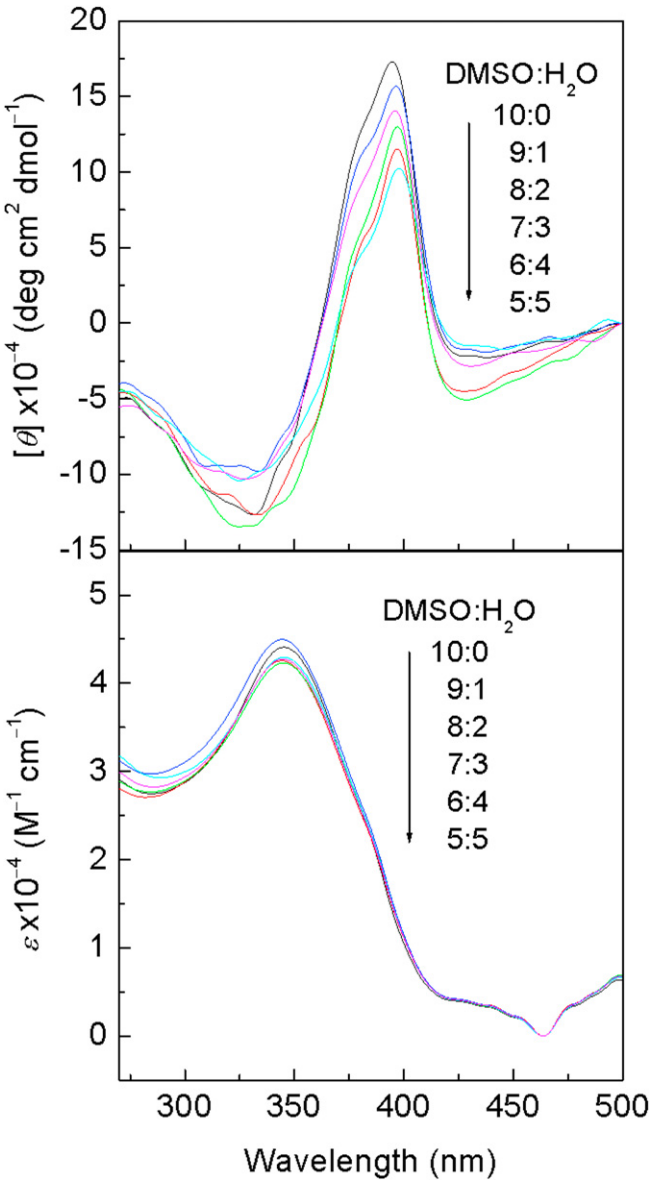


Fig. 2. CD and UV–vis spectra of poly(**1**) measured in DMSO/H_2O with various compositions ($c = 4.5 \times 10^{-5}$ mol L^{-1}) at room temperature. Sample: run 1 in Table 1.

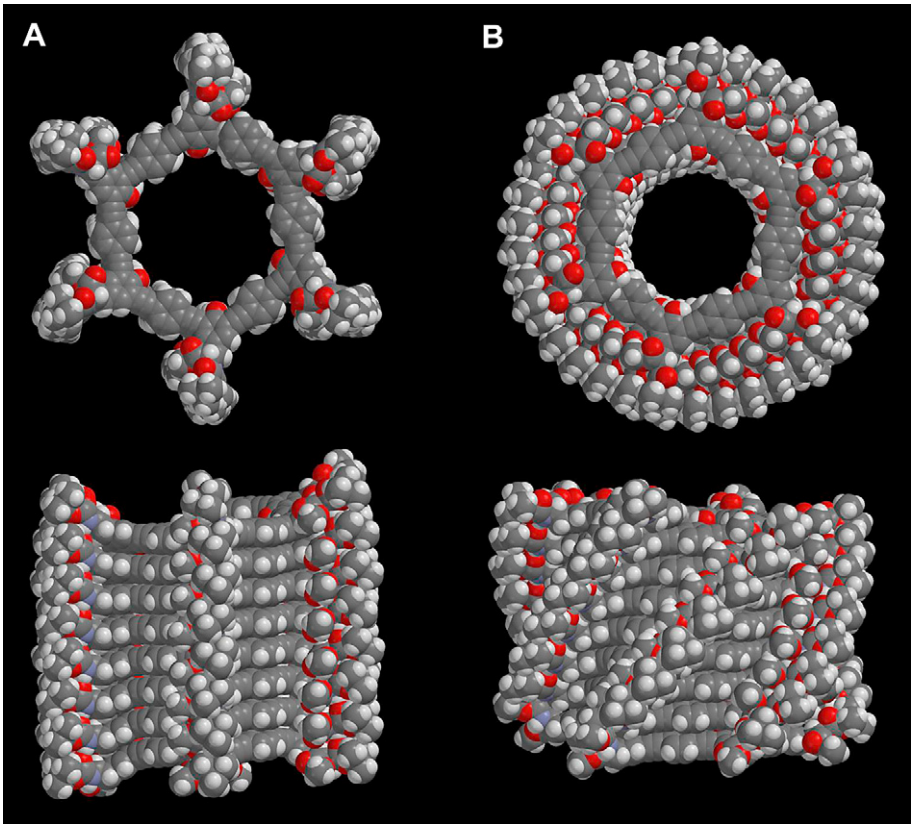


Fig. 3. Top and side views of possible conformers of poly(**1**) (48-mer) optimized by MMFF94. A: Helix with 6 monomer units per turn. B: helix with 5.8 monomer units per turn.

chains of conjugated helical polymers become irregularly twisted, the ε values decrease because the conjugation length becomes short [11]. It is therefore assumed that poly(**1**) decreased one-handedness of screw sense, almost keeping the total helix content upon addition of H_2O.

We also measured the CD and UV–vis spectra of poly(**2**) in CH_2Cl_2, THF, and DMSO to find no CD signal. The phenolic hydroxy groups play an important role for poly(**1**) to take a helical structure with biased screw sense. It is not clear which of a random conformation and a helical one with the same amount of right- and left-handed screw senses poly(**2**) adopts. Poly(**2**) exhibited a UV–vis absorption peak around 330 nm, which is 20 nm shorter than that of poly(**1**). Since both **1** and **2** show the λ_{max} at 285 nm, poly(**2**) seems to have a conjugation length shorter than that of poly(**1**), suggesting the existence of a random conformation. However, the coexistence of right- and left-handed helices with a 1:1 ratio is not deniable.

Intramolecular hydrogen bonding is used to stabilize helical structures not only of biopolymers such as protein and DNA but also of some artificial π-conjugated polymers, which include poly(phenylacetylene)s substituted with hydroxy groups [12], poly(*N*-propargylamide)s [13], and poly(*N*-propargylcarbamate)s [14]. In a similar fashion, poly(**1**) and poly(**2**) may form intramolecular hydrogen bonding between the carbamate groups. We measured the solution-state IR spectra of the polymers to check this possibility. As a result, no difference was found in the $V_{C=O}$ values of carbamate and ester groups from those of **1** and **2** in $CHCl_3$, THF, and DMSO (See supplementary data). Thus it is concluded that the carbamate and ester groups of the present polymers do not form intramolecular hydrogen bonding, which is different from the aforementioned polymers.

Amphiphilicity is an important driving force in guiding secondary and tertiary structures of proteins [15]. In a similar fashion, *meta*-linked poly(phenyleneethynylene)s having hydrophilic side groups such as oligo(ethylene glycol) chains, amine, sulfonate and carboxylate ionic side groups take a helical conformation driven by amphiphilicity as described in the Introduction [3,6]. The phenolic hydroxy groups of poly(**1**) are hydrophilic, while the ester and Boc groups are hydrophobic compared to the hydroxy groups. It is likely that poly(**1**) forms a helical structure due to the amphiphilic character. It seems that the amphiphilic character of poly(**2**) having ether-protected hydroxy groups is not enough to drive poly(**2**) to take a helical conformation.

3.3. Conformational analysis of poly(*1*)

Thus far, the conformations of poly(*m*-phenyleneethynylene)s have been analyzed in detail. Amine- [16] and ester-functionalized [17] poly(*m*-phenyleneethynylene)s are suggested to prefer helical conformations to coiled and extended ones in water, wherein surrounding water molecules play an important role to fold the polymer chains. This is understood from the fact that the helical structure maximizes interactions between the polar side chains and solvents, and also π-stacking interaction. Namely, the helical structure minimizes unfavorable contacts between the hydrocarbon backbone and polar solvents as well [1b].

Fig. 3 shows possible conformers of poly(**1**) (48-mer), whose geometries were optimized by the molecular mechanics method (MMFF94) [18]. It seems that poly(**1**) takes a helical conformer with 6 monomer units per turn (A in Fig. 3), because a folded *m*-PE-*p*-PE linkage theoretically forms a regular hexagon. However, this helical structure is as large as 21.7 kJ/(mol unit) unfavorable than a helix with 5.8 monomer units per turn (B in Fig. 3) due to the steric repulsion between the eclipsed substituents on the phenylene rings at *i* th and $(i+6)$th monomer units. Poly(**1**) is likely to take an

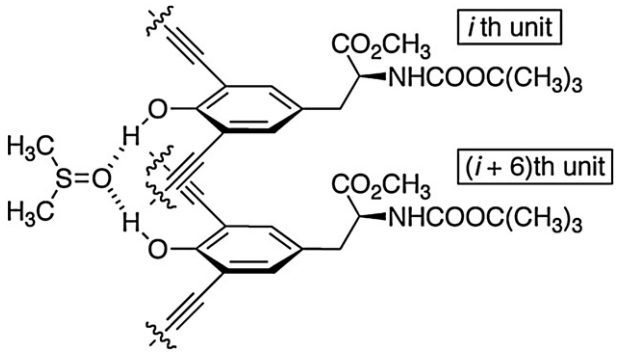


Chart 2. Possible DMSO-assisted hydrogen bonding between the hydroxy groups at *i* th and $(i + 6)$th units of poly(**1**).

incompletely regular hexagonal helix structure like B, in which the substituents on the phenylene rings at *i* th and $(i + 6)$th monomer units are not eclipsed. The diameter of helix B is 24.0 Å, which is much larger than that of poly(*m*-phenyleneethynylene)s (12.0–14.4 Å) [17] due to the presence of *p*-phenylene linkages. The helical pitch is 4.1 Å, which is consistent with the turns of the helix being near van der Waals contact and comparable to π-stacking between aromatic rings [17].

The distances between the hydroxy oxygen at *i* th unit and hydroxy hydrogen at $(i + 6)$th units of helices A and B are 3.1 and 3.5 Å, respectively, both of which are too long to form hydrogen bonds. As described above, poly(**1**) forms a helical structure efficiently in DMSO that possibly participates in hydrogen bonding, while it does not in CH_2Cl_2 that has no hydrogen bonding ability. It should be noted that poly(**2**) having methyl ether-protected hydroxy groups does not form a helix. These results can be explained by considering the formation of hydrogen bonds between hydroxy groups assisted by DMSO as illustrated in Chart 2. In the case of helix B, the distance between hydrogen atoms of hydroxy groups and oxygen atom of DMSO is 1.8 Å, when they form hydrogen bonds as shown in Chart 2. This is acceptable from the literature regarding DMSO-hydrogen bonds [19]. It is likely that the DMSO-assisted hydrogen bonding contributes to stabilize the helical structure of poly(**1**), and the lack of this contribution causes no helix formation of poly(**2**).

It is considered that the bisignated CD signals at 390 and 330 nm of the polymer in DMSO (Fig. 1) are caused by exciton chirality based on the cooperative interaction between the main chain chromophore [20], because the λ_{max} of the UV–vis absorption is positioned at the inflection point (350 nm) of the plus and minus CD signals. Judging from the positive first and negative second Cotton effects together with the molecular modeling, the helical sense is considered to be right-handed as illustrated in Fig. 3.

3.4. Conformational change of poly(*1*) upon addition of base

The helical conformation of hydroxyphenylglycine-based poly(*m*-phenyleneethynylene-*p*-phenyleneethynylene)s (Chart 1) is stable to addition of alkali metal hydroxides, although the polymers have phenolic hydroxy groups that are susceptible to base [8]. This is presumably due to the presence of intramolecular hydrogen bonding between the amide groups, which effectively stabilizes the helical structure. Since poly(**1**) in the present study takes a helical conformation without the assistance of such intramolecular hydrogen bonding, it is forecasted that poly(**1**) changes the conformation according to the addition of base more largely than the polymers illustrated in Chart 1. Fig. 4 depicts the effect of NaOH addition on the CD and UV–vis spectra of poly(**1**) measured in

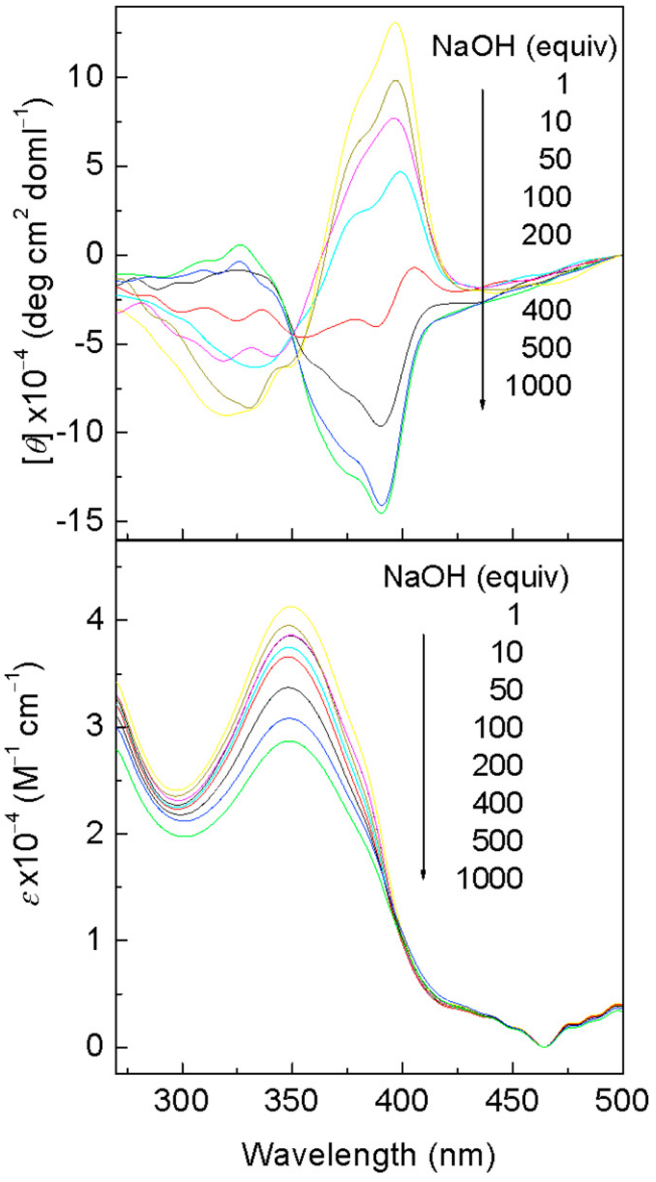


Fig. 4. CD and UV–vis spectra of poly(**1**) upon addition of NaOH measured in DMSO/H_2O = 9/1 (v/v, $c = 4.5 \times 10^{-5}$ mol L^{-1}) at room temperature. Sample: run 1 in Table 1.

DMSO/H_2O = 9/1 (v/v). The CD spectroscopic pattern was very sensitive to NaOH as was predicted. The plus-signed CD signal around 390 nm gradually decreased the intensity by raising the amount of NaOH, and almost disappeared at 200 equiv. Further addition of NaOH brought about appearance of a minus-signed CD signal at 390 nm, and increase of intensity. These results suggest

Table 2
Alkaline hydrolysis of poly(**1**) and poly(**2**)[a].

Polymer	Yield[d] (%)	M_n[e]	M_w/M_n[e]
Poly(**1**)[b]	62	6600	3.73
Poly(**2**)[c]	64	7500	3.81

[a] Conditions: in DMF/H_2O = 1/1 (v/v), 50 °C, 3 h.
[b] Sample: run 1 in Table 1.
[c] Sample: run 3 in Table 1.
[d] H_2O-insoluble part.
[e] Determined by GPC eluted with LiBr solution in DMF (10 mM) calibrated by polystyrene standards.

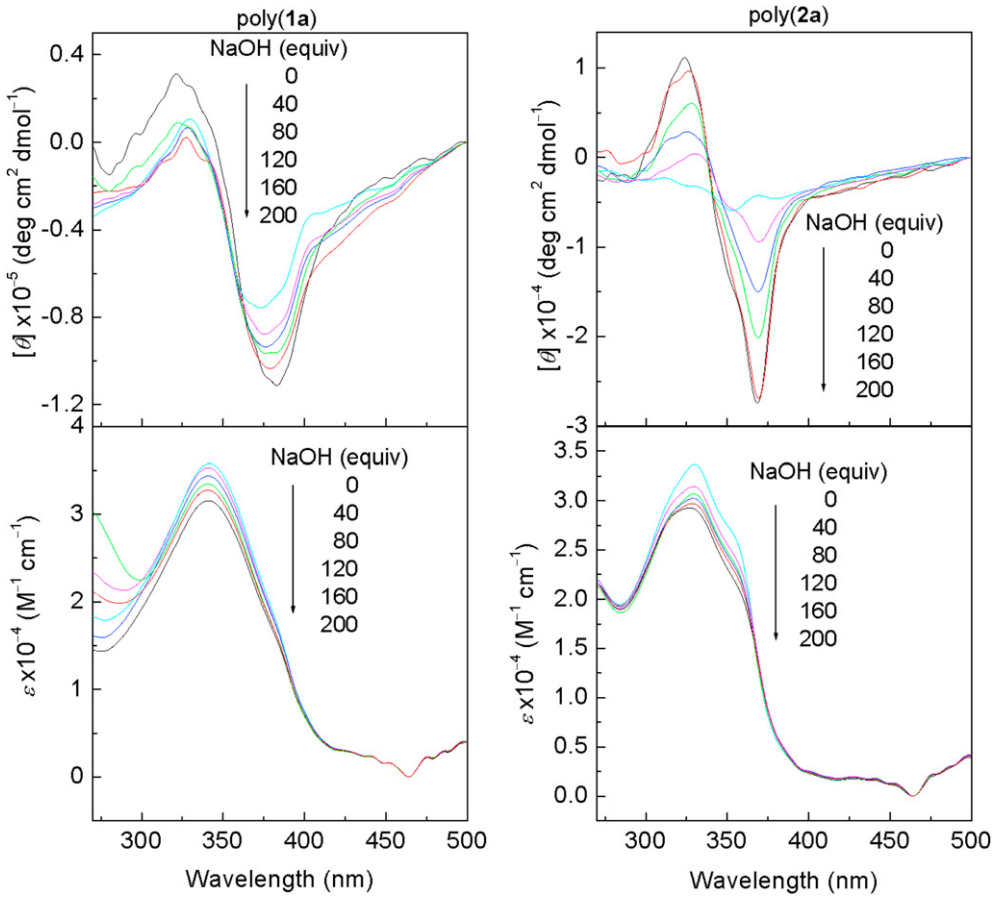


Fig. 5. CD and UV–vis spectra of poly(**1a**) and poly(**2a**) upon addition of NaOH measured in MeOH/H_2O = 1/1 (v/v, $c = 5.0 \times 10^{-5}$ mol L^{-1}) at room temperature.

that poly(**1**) transformed the predominant screw sense upon NaOH addition [21]. Since the spectra are close to mirror-images before and after 1000 equiv of NaOH addition, it is considered that the increasing amount of NaOH yields an excess of the opposite-handed helix, which is very similar in structure to and in equilibrium with the original one. An isodichroic point is observed at the maximum UV absorption. So evidently the base plays a role in the transfer of chirality from the tyrosine side groups to the main chain secondary structure. It is likely that other alkali metal hydroxides also interact with poly(**1**) to affect the conformation. We also measured the CD and UV–vis spectra of poly(**1**) in the presence of LiOH, KOH, CsOH, and RbOH as well as NaOH to find no clear difference and tendency among these metal hydroxides (See supplementary data).

3.5. Hydrolysis of the ester groups of the polymers

We further examined the hydrolysis of methyl ester moieties of poly(**1**) and poly(**2**) using NaOH. The polymers satisfactorily underwent alkaline hydrolysis to give the corresponding polymers [poly(**1a**) and poly(**2a**)] having carboxy groups in good yields as listed in Table 2. The GPC-determined M_n's of the hydrolyzed polymers were lower than those of the polymers before hydrolysis. This is partly because of removal of methyl groups from the side chains, and probably the large interaction between the pendent carboxy groups and polystyrene gels as well, resulting in the long elution times. The polymers were soluble in polar solvents such as DMF, DMSO, MeOH, and basic water.

3.6. Chiroptical properties of poly(*1a*) and poly(*2a*)

Fig. 5 depicts the effect of NaOH addition on the CD and UV–vis spectra of poly(**1a**) and poly(**2a**) in MeOH/H_2O = 1/1 (v/v). It should be noted that poly(**1**) showed a plus-signed first Cotton signal, while poly(**1a**) showed a minus-signed one. This indicates that the polymer kept a helical form after hydrolysis but changed the predominant screw sense. On the contrary, poly(**2a**), obtained by the hydrolysis of poly(**2**), exhibited no CD signal similar to the prepolymer [poly(**2**)], indicating that neither of them forms a predominantly one-handed helical conformation irrespective of the substituents (carboxy and ester groups). It seems that the phenolic hydroxy groups play a more important role on formation helix than carboxy groups. Addition of NaOH resulted in increase of CD intensity of poly(**1a**), and induction of CD signals in the case of poly(**2a**). It is considered that NaOH ionized the carboxy groups to increase the polarity, leading to folding into a predominantly one-handed helical structure. The helical structure of poly(**2a**) was more sensitive to base than that of poly(**1a**). Since the phenolic hydroxy groups of poly (**1a**) are effective to induce a predominantly one-handed helix as described above, it can form a helix even in the absence of NaOH, and therefore the responsiveness seems to be small. On the other hand, poly(**2a**) cannot form a predominantly one-handed helix in the absence of NaOH, probably because the phenolic hydroxy groups are protected. By the addition of base, poly(**2a**) acquires highly polar carboxylate groups, which seem to be effective to induce a predominantly one-handed helical structure.

Fig. 6 depicts the dependence of CD and UV–vis spectra of poly (**1a**) and poly(**2a**) on the composition of MeOH/H_2O. Interestingly,

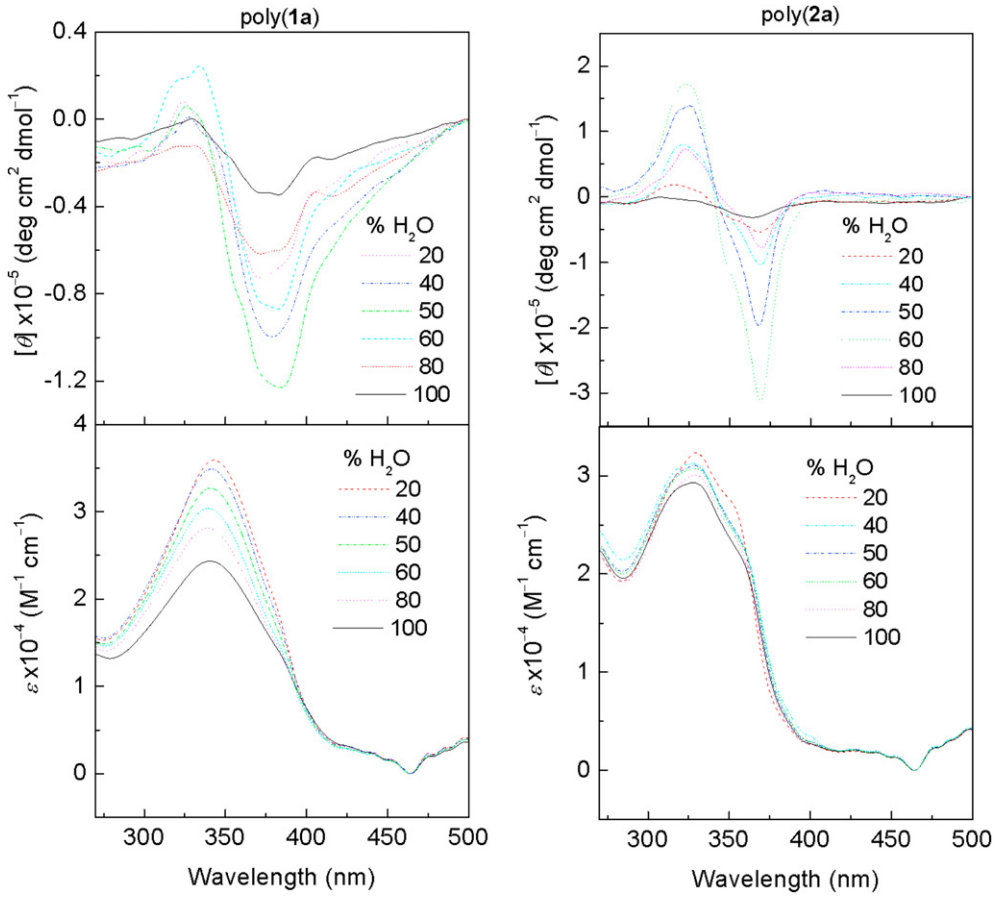


Fig. 6. CD and UV–vis spectra of poly(**1a**) and poly(**2a**) measured in MeOH/H_2O with various compositions ($c = 5.0 \times 10^{-5}$ mol L^{-1}, $c_{NaOH} = 0.05$ M) at room temperature.

the amplitude of CD signal of the polymers varied with the solvent composition. Poly(**1a**) and poly(**2a**) increased the CD intensity with increasing water content to reach the maxima at 50 and 60 volume % water, respectively. This result indicates that the hydrophobic effect is important for the polymers to adopt a helical conformation to a greater extent [6b]. Further addition of H_2O resulted in a decrease in the amplitude of the CD signal.

4. Conclusion

In this paper, we have demonstrated the Sonogashira–Hagihara polymerization of 3′,5′-diiodo-*N*-α-*tert*-butoxycarbonyl-L-tyrosine methyl ester (**1**) and 3′,5′-diiodo-*N*-α-*tert*-butoxycarbonyl-*O*-methyl-L-tyrosine methyl ester (**2**) with *para*-diethynylbenzene (**3**). The polymerization satisfactorily proceeded to give the corresponding poly(*m*-phenyleneethynylene-*p*-phenyleneethynylene)s [poly(**1**) and poly(**2**)]. Poly(**1**) took a predominantly one-handed helical conformation in DMSO, but poly(**2**) did not. It is considered that the phenolic hydroxy groups play an important role to induce the regulated structure of poly(**1**); the helical conformation of poly (**1**) is induced by the amphiphilic character. Poly(**1**) inverted the predominant screw sense in DMSO/H_2O = 9/1 (v/v) by the addition NaOH. Poly(**1**) and poly(**2**) were satisfactorily converted into the corresponding polymers having carboxy groups [poly(**1a**) and poly (**2a**)] by alkaline hydrolysis of the ester groups. The polymers changed the helical structures responding to base and MeOH/H_2O compositions, especially the case of poly(**2a**) having protected phenolic hydroxy groups. The results obtained in the present research together with those of hydroxyphenylglycine-based polymers (Chart 1) [8] have developed a new way of molecular design of helical poly(phenyleneethynylene) derivatives having phenolic hydroxy groups.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the on-line version, at doi:10.1016/j.polymer.2010.03.048.

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