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Ultrastrong, Transparent Polytruxillamides Derived from Microbial Photodimers

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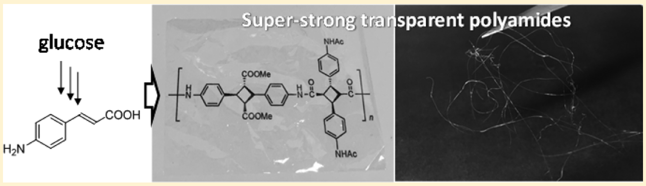
Supporting Information



ABSTRACT: Ultrastrong and transparent bioplastics are generated from fermented microbial monomers. An exotic aromatic amino acid, 4-aminocinnamic acid, was prepared from a biomass using recombinant bacteria, and quantitatively photodimerized, and diacid and diamino monomers that were both characterized by a rigid α-truxillate structure were generated. These two monomers were polycondensed to create the polyamides with a phenylenecyclobutane repeating backbone such as poly{(4,4′-diyl-α-truxillic acid dimethyl ester) 4,4′-diacetamido-α-truxillamide} which was processed into amorphous fibers and plastic films having high transparency. In spite of noncrystalline structure, mechanical strength of the fiber is 407 MPa at maximum higher than those of other transparent plastics and borosilicate glasses, presumably due to the tentative molecular spring function of the phenylenecyclobutanyl backbone.

INTRODUCTION

Weight saving of industrial materials is indispensable for establishment of sustainable societies. Utilization of plastics instead of hard and heavy materials such as metals and glasses is very effective on weight saving. Most plastics, however, have less thermomechanical performances than the heavy materials.[1] Especially, transparent plastics such as polycarbonates are expected to alternate glass materials, but their thermomechanical performances were too low to apply in wide fields of electronics and optics. We have synthesized functional aromatic polymers[2−4] and recently produced the functional polyimide films[5] with good transparency and very high thermoresistance from the less toxic aromatic monoamine 4-aminocinnamic acid (4ACA, compound **1**) using genetically engineered microbial cells from 4-aminophenylalanine, which is an intermediate of chloramphenicol synthesis.[5] Other researchers have also prepared transparent polymers with high thermoresistance. However, their mechanical strengths were not high enough to alternate conventional heavy inorganic materials. This should be attributed to a serious dilemma in molecular design that amorphous structure is indispensable for the good transparency but effectively reduces the mechanical strengths.

Here we overcome the dilemma using truxillamide backbone comprising rigid phenylenes and their connecting cyclobutanes (CB) behaving as a molecular spring showing possible tautomerization to produce ultrastrong and transparent polyamides having higher mechanical strength than heavy materials such as glasses and even gold, silver, copper, and so forth. Our improvement of fermentation efficiency of 4ACA is of advantage for producing the biopolyamides in a large scale.

RESULTS AND DISCUSSION

Syntheses. The dianiline monomers of the aimed polyamides were synthesized by an analogous method to the previously reported polyimide syntheses,[5] where natural 4-aminophenylalalnine was bioconverted to 4ACA at a low efficiency of 0.13 g/L, and a direct photoirradiation of its hydrochloride salt (compound **2**) powder without agitation generated a milligram scale of the 4ACA dimer (compound **3**). Here, we biosynthesized 4ACA by using glucose biomass as a starting material at a remarkably improved efficiency of 2.2 g/L (detailed data sets are summarized in the Supporting Information) and made a 100 g scaled synthesis of the photodimer **3** in a benzene-dispersion state under magnetic agitation. The three-dimensional structure deduced from X-ray diffraction of the single crystal showed that the main backbone

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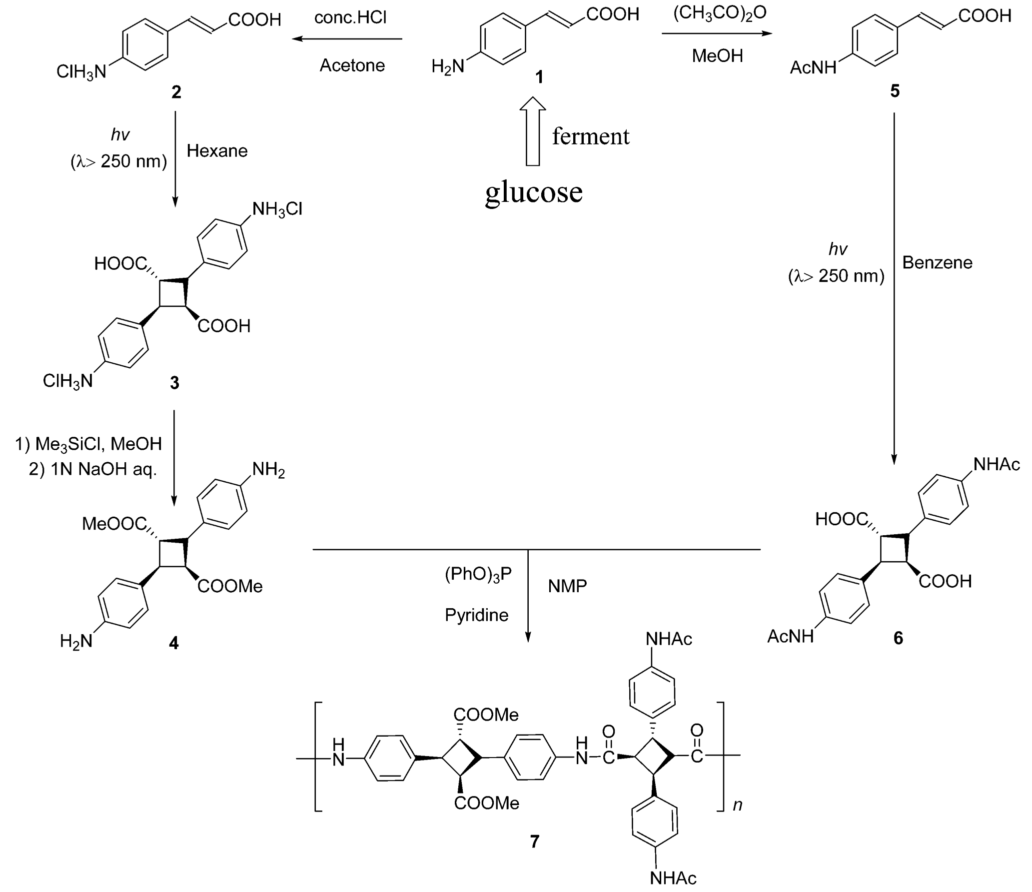
Scheme 1. Synthetic Route of Bio-Based Aromatic Polyamide from 4-Aminocinnamic Acid via Two α-Truxillic Acid Derivatives



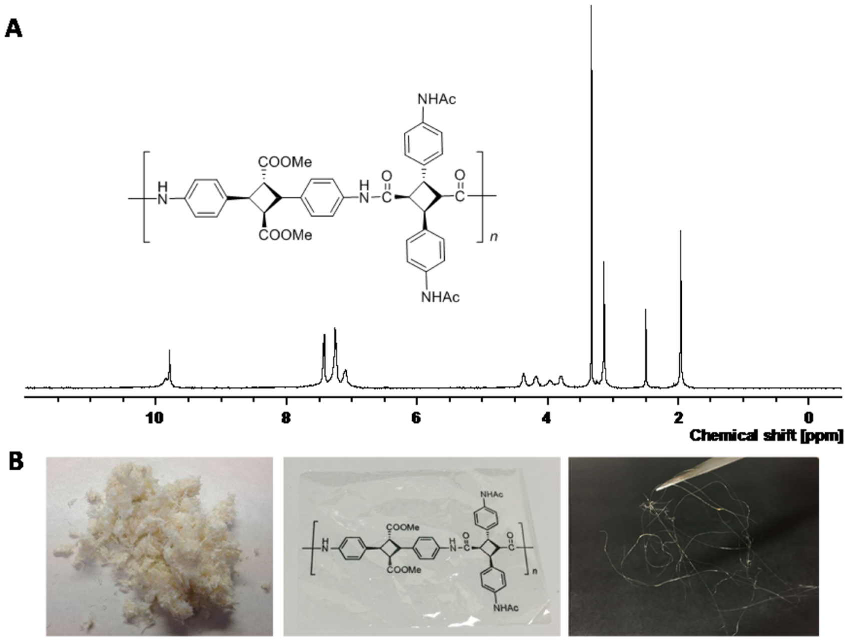


Figure 1. Structural analysis and pictures of bio-based aromatic polyamide 7. (A) ^{1}H NMR spectrum of aromatic polyamide 7. (B) Left, as-prepared fibrils; center, cast film; right, spun fiber.

comprised an α-truxillic acid group formed as a result of head-to-tail antiaddition of two cinnamoyls, and the phenylenes and carboxyls were oppositely oriented (Figure S3). Following methylation was made by the procedure shown in the previous report to create 4,4′-diamino-α-truxillic acid dimethyl ester (compound **4**). These photochemical and chemical procedures were quite simple and yielded over 90 wt % of aromatic diamine **4** from **1**.

In order to synthesize the diacid monomer, we acetylated **1** to give compound **5** and then investigated its photodimeriza-

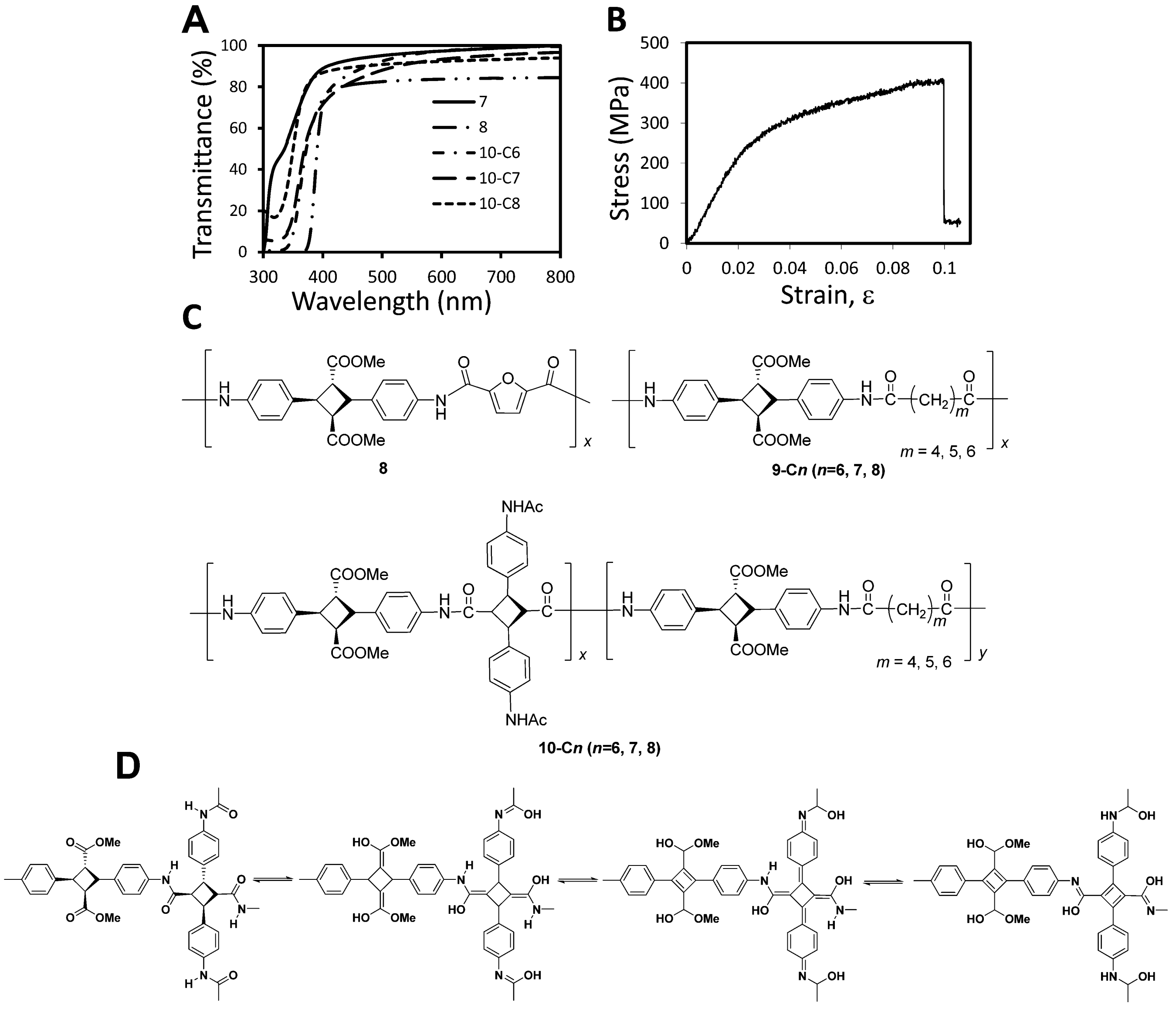


Figure 2. Transparency and mechanical properties of prepared polyamides. (A) Transmittance curve of polymers **7**, **8**, and **10**. (B) Representative stress−strain curve of polymer **7**. (C) Structures of polymers **8**, **9**, and **10**. (D) Tentative tautomerization of cyclobutanedioyl-4,4′-truxillamide backbone.

tion in various organic solvents (Table S6). The photoreaction in dispersions of benzene and dichloromethane resulted in the formation of 4,4′-diacetoamido-α-truxillic acid (compound **6**) with a perfect yield. On the other hand, photoirradiation in solutions of acetonitrile and DMSO, which were both good solvents for **5**, produced its *cis*-isomer. Schmidt's rule states that [2 + 2] photocyclization reactions occur in the solid state, since the distance between molecules in the double bond should be <4.2 Å.[6,7] This explains why the homogeneous solution of **5** in acetonitrile or DMSO did not create the targeted aromatic diacid **6**, which had two carboxyl groups that were used as a diacid monomer for polyamide synthesis without further chemical manipulation. The yield in the overall chemical processes was over 80 wt %. Thus, the aromatic diacid **6** and diamine **4** were synthesized from bioderived compound **1** in a high efficiency.

A bio-based aromatic polyamide, poly{(4,4′-diyl-α-truxillic acid dimethyl ester) 4,4′-diacetamido-α-truxillamide} (compound **7**), was synthesized from **4** and **6** via the procedures shown in Scheme 1. The polyamide synthesis route from only one bio-based amino acid **1** via two different dimerizations to successive polycondensation of these two monomers had an advantage in terms of bioresource efficiency. The polycondensation of monomers **4** and **6** catalyzed by conventional triphenyl phosphite and pyridine produced a polyamide **7**, whose structure was confirmed by the ^{1}H NMR study (Figure 1A). The estimated number- and weight-average molecular weight of **7** determined by gel permeation chromatography (GPC) were 1.0×10^4 and 2.1×10^4, respectively. This polyamide is notable since the repeating unit should be regarded as a tetramer of **1** and modified by bioavailable acetic acid and methanol.

Thermomechanical Properties and Transparency. The polyamide was soluble in aprotic polar solvents, and a transparent film and fiber were easily prepared from polymer **7** in dimethylformamide (Figure 1B). The wavelength dependence of transparency is shown in Figure 2A showing high transparency 89% at 400 nm, which is comparable to those of

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Table 1. Mechanical and Thermal Properties of Aromatic Polyamides and Other Materials

materials	M_n ($\times 10^{-4}$)[a]	M_w ($\times 10^{-4}$)[a]	PDI[a]	T_{d10}[b] (°C)	T_g[c] (°C)	Young's modulus, E_{init}[d] (GPa)	strength, σ[d] (MPa)	elongation at break, ε[d] (%)	transparency[e] (%) 400 nm	transparency[e] (%) 450 nm
7	1	2.1	2.06	370	273	11.4 ± 1.6	356 ± 33	7.2 ± 3.7	89 (336 nm)	93
8	2.7	5.4	1.99	355	198	8.0 ± 0.3	163 ± 71	13.6 ± 6.8	71 (391 nm)	81
9	4.1−5.4	5.1−9.1	1.24−1.69	365−370	151−159	ND	ND	ND	ND	ND
10-C6	5.5	6.5	1.18	359	243	12.1 ± 4.1	407 ± 188	36.9 ± 19.1	74 (373 nm)	87
10-C7	5	6.2	1.26	358	250	10.7 ± 1.0	284 ± 42	17.3 ± 7.6	71 (370 nm)	83
10-C8	2.5	2.9	1.9	356	252	8.7 ± 1.0	223 ± 36	7.7 ± 2.7	87 (350 nm)	90
polyamide **11**	2.5	6.5	2.6		29	1.3	67			
fluoropolyimide **1**[f]					292	3.3	114		86	87
fluoropolyimide **2**[g]					309	2.4	129		1	17
fluoropolyimide **3**[h]					327	2.9	122		76	87
polycarbonate	1.2	3.1	2.64		150	1.9	62	200	87	89
PMMA	5.9	9.3	1.58		121	2.3	60	3.1	85	90
ZEONEX					140	2.4	63		90	91
nanocellulose						13	223		88 (ca. 310 nm)	90

[a] The weight-average molecular weight, M_w, the number-average molecular weight, M_n, and the distribution of polymer molecular weight, PDI, of polyamides were measured by GPC. [b] 10% weight loss temperature, T_{10}, was obtained from TGA curve scanned at a heating rate of 10 °C/min under a nitrogen atmosphere. [c] Glass transition temperature, T_g, which was measured by DSC thermogram scanned at a heating rate of 10 °C/min under a nitrogen atmosphere. [d] Tensile strength at break, Young's moduli, and elongation at break were obtained from tensiometer at room temperature. ND refers to not determined. [e] Transmittances were measured as transparency by UV/vis spectrophotometer. UV cutoff wavelengths, λ_0, meaning the wavelength at 50% transmittance are shown in parentheses. [f] Fluoropolyimide **1** refers to the polyimides derived from hexafluoroisopropylidene diphthalic anhydrides and 2,5-diamino-2,5-dideoxy-1,4:3,6-dianhydrosorbitol.[19] [g] Fluoropolyimide **2** refers to the polyimides derived from 1,4-bis(3,4-dicarboxytrifluorophenoxy)tetrafluorobenzene dianhydride and tetrafluoro-*m*-phenylenediamine.[19] [h] Fluoropolyimide **3** refers to the polyimides derived from 2,2-bis(3,4-dicarboxyphenyl)hexafluoropropane dianhydride and 2,2′-bis(trifluoromethyl)-4,4′-diaminobiphenyl.[20]

conventional and newly developed transparent resins (Table 1). Cutoff wavelength was in the range of ultraviolet region. The thermogravimetry revealed no weight loss at temperatures below 300 °C and 10% weight loss at 367 °C (weight loss temperature, T_{d10}). The T_{d10} value of polymer **7** was not so high because of the methyl ester moiety limits their thermal degradation properties. Differential scanning calorimetry (DSC) showed that the glass transition temperature, T_g, of the polymer was 273 °C. These thermal properties are lower than those of previous biopolymers[2] but are higher than those of bio-based plastics reported other researchers. The both values of T_g and T_{10} were sufficient for application of polymer **7** as an industrial plastic with high thermoresistant performance.[8] The stress−strain curve of polymer **7** was also investigated (Figure 2B), and the maximum tensile strength and Young's modulus were $\sigma = 356 \pm 33$ MPa and $E = 11.4 \pm 1.6$ GPa, respectively ($n = 5$). The σ value of polymer **7** was much higher than those of transparent resins such as conventional polycarbonate, poly(ethylene terephthalate), poly(vinyl acetate), and poly(methyl methacrylate) ranging 62, 73, 50, and 60 MPa, respectively,[9,10] and recently developed transparent resin, ZEONEX, showing 63 MPa.[11] Notably, σ of polyamide **7** was also much higher than those of conventional bio-related polyamides such as polyamide 11 (PA11)[12,13] and of protein fibers such as collagen, keratin, elastin, and titin.[14−18] According to recent scientific reports on transparent resins, fluorinated aromatic polyimides show high σ values ranging 114−129 MPa[19,20] and bio-derived films of nanocelluloses show 223 MPa,[21] but the present biopolyamides showed much higher values than them. Furthermore, mechanical strength of the polyamide **7** exceeded those of borosilicate glasses (40−100 MPa).[22] The polyamide **7** was amorphous as a result of wide-angle X-ray studies (representative X-ray diffraction diagram is shown in Figure S5) while their mechanical strengths were very high in spite of no reinforcement by crystalline domains. Such high mechanical strength is presumably derived from the adequate deformation of CB ring with a nonplanar structure just like as a V-shaped spring to avoid the stress concentration during elongation. Additionally, the backbones of cyclobutanedioyl-4,4′-truxillamide have a rigid tautomerized structure with coplanarity drawn on the right of Figure 2D. If the coplanar structure was formed under mechanical stress, the most stressed parts of the macromolecular chains could be quite rigid to tolerate against the stress-focusing, and the original softer chains around this parts could be deformed to impart the toughness to the materials. In order to confirm the mechanochemical change of polymer backbones, we made IR/ATR studies on the polyamide film under strong pressure and found a small change in IR peak around 1550 cm^{-1} which was assigned to overlapped vibrations of amide II and carbon−carbon double bond stretching. This change suggests that the mechanical stress to the film can affect the structural change of amide and benzenes, possibly relating with proposed tautomerization.

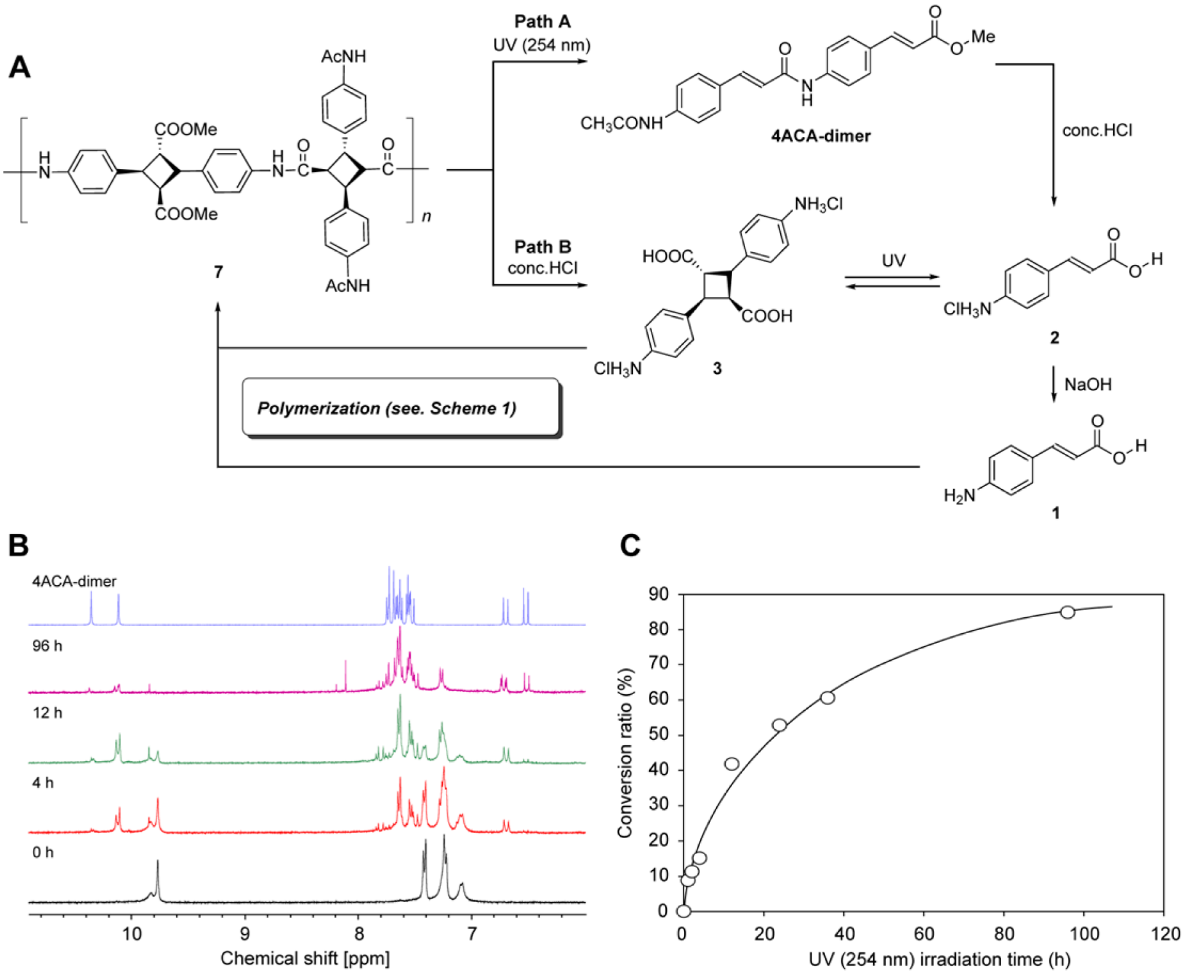

Figure 3. Photonic and hydrolytic degradation of polyamides. (A) Chemical pathways of degradation. (B) Change of ^{1}H NMR spectra of polyamide 7 by photoirradiation. (C) UV-irradiation time dependence of conversion ratio into 4ACA dimer from polyamide 7.

We assessed polycondensation of the bio-based aromatic diamines **4** with bio-derived diacids including aliphatic diacids (dicarboxylic derivatives of fatty acids) and 2,5-furandicarboxylic acid. The direct amidation of compound **4** and the synthesis of poly{(4,4′-diyl-α-truxillic acid dimethylester) 2,5-frandicarboxylamide} (**8**) from 2,5-furandicarboxylic acid proceeded as described in the experimental section (Supporting Information).

The syntheses of aromatic−aliphatic poly{(4,4′-diyl-α-truxillic acid dimethyl ester) α,ω-alkyloylamide} (**9**) where alkyloyls are hexyloyl (C6), heptyloyl (C7), and octyloyl (C8)) polyamides using compound **4** with aliphatic diacids proceeded using the corresponding linear alkylene carboxylic chlorides (Figure 2C). Table 1 summarizes the thermomechanical properties of these polyamides. The tensile strength and Young's modulus of polyamide **8** were lower than those of polyamide **7** and polyamides **9** in any carbon number of aliphatic chains showed mechanical brittleness, either. As a consequence, the double α-truxillic moiety comprising cyclobutanedioyl-4,4′-truxillamide was indispensable for inducing high mechanical strength, which supports the above-mentioned effects of tautomerized coplanar structures on the mechanical strength increase (Figure 2D).

We then attempted to synthesize terpolyamides using third monomers with aliphatic chains (**10-C*n***) (*n* refers to the number of carbon atoms in the aliphatic diacids), with keeping double α-truxillic moiety in the backbone. The structure and compositions of terpolymers were determined by NMR spectroscopy that were nearly equal to in-feed composition of CB (x) and alkylene (y) moiety as shown in the Supporting Information. As a result of mechanical test, these terpolyamides **10-C6**, **10-C7**, and **10-C8** kept high mechanical strength (stress−strain curves are shown in the Supporting Information). The incorporation of aliphatic chains increased elongation at break with keeping high values of σ and E. In addition, the elongation values of these terpolymides were decreased with increasing in the carbon number of aliphatic groups, which might be attributed to the entanglement more efficiently induced by the longer alkyl chains. The results indicate the good acceptability of cyclobutanedioyl-4,4′-truxillamide structure for third components, which is very important to control the physical properties by the additional third component and/or to impart some functions of the third component.

Degradation of Fully Bio-Based Aromatic Polyamide 7. The degradability of bio-derived polymers into monomers is important in terms of sustainability because it enables their chemical recycling. No enrichment culture using the powdered polyamide **7** as a sole carbon source resulted in microorganism that grew under the conditions. No soil bacteria were obtained by streaking soil suspension on plates that degrades polyamide **7**. These results indicated that the polymer is stable in nature although this conclusion awaits future investigation on the polyamide-degrading microorganisms. A system comprising hydrolysis and photodegradation was tested to accomplish this since polyamide 7 had such a chemically stable backbone with aromatic rings and amide bonds that typical hydration methods such as biodegradation would be ineffective. This degradation system was predicted to generate compound **1** from 7.

Since polyamide 7 has CB rings cleavable by UV-irradiation, photodegradation of the polymer backbone was carried out as shown in path A of Figure 3A. If the photodegradation reaction of polyamide **7** proceeds quantitatively, 4ACA dimer shown in path A of Figure 3A should be formed. By UV-irradiation with a main wavelength of 254 nm, broad signals assigned to phenylenes and CB of polyamide **7** around 7.00−7.50 and 3.68−4.42 ppm, respectively, became weaker with longer irradiation time. After 36 h irradiation or longer, the NMR spectrum of polyamide **7** was overlapped by signals appearing at 6.71 and 6.53 ppm assigned to allylic protons on *trans*-vinylene structure of 4ACA-dimer (top of Figure 3B) which was synthesized by a condensation of 4ACA methyl ester and compound **5**. After UV irradiation for 96 h, the conversion ratio attained to 84.9% calculated from peak area of allylic and aromatic protons as shown in Figure 3C. In addition, GPC chromatography shown in Figure S6 demonstrated the decrease in the polyamide molecular weight into 670 g/mol, which demonstrated photodegradation of polyamide **7** into oligomers.

Besides the polyamide **7** powders were effectively hydrolyzed in concentrated HCl at reflux temperature for 8 h (path B of Figure 3A) (NMR in Supporting Information), and the needle crystal appeared by successive cooling. The recovery rate of **3** was 97 wt % vs **7**, and the mass spectrum of the product confirmed its high purity. 4ACA was then obtained by photocleavage of the CB ring of recovered compound **3** via 1 h irradiation of a Xe lamp whose wavelength was filtrated around a 254 nm by band-pass filter. We then polymerized the recovered resource **2** to get polyamide **7** again.

Thus, we developed novel ultrahigh strong, transparent bioplastics which could be versatile for application in the manufacture of automobile parts, flexible electronic papers, flexible panels for solar cells, printable electronic circuit, sensor lenses, and other various materials of optical devices. The photodegradability of the present bio-based polyamide supported the monomer recycling system which is indispensable for sustainable society.[23]

CONCLUSION

We established microbial conversion system from sugar to 4ACA using the selected isozyme of phenylalanine ammonia lyases to prepare aromatic monomers, both aromatic diamines, and diacids as photodimers of 4ACA derivatives. As a result of polycondensation of the bio- and photoderived monomers, we successfully synthesized fully bio-based aramid polymers with a truxillamide backbone. The tensile strength, Young's modulus, and transparency of the polyamide were 356 ± 33 MPa, 11.4 ± 1.6 GPa, and 93%, respectively, which were all higher than those of a widely used transparent plastics. Especially the mechanical strength of the fiber is 407 MPa at maximum higher than those borosilicate glasses, presumably due to the molecular spring function possibly showing tautomerization in the phenylenecyclobutanyl backbone—in spite of non-crystalline structure. In addition, the polyamide was efficiently degraded into monomer which was successfully repolymerized in high yield. Thus, we developed high-performance and recyclable superengineering bioplastics which was first prepared and would be widely applied in materials of automobile parts, medical instruments, and electronic devices.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications Web site. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.6b00220.

Experimental section; Figures S1−S9 and Tables S1−S6 (PDF)

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Author Contributions

S.T. and S.M. made equal contributions. T.K. and N.T. designed studies and prepared the manuscript with S.T. and S.M. S.M., S.Z., and Z.Z. designed and performed biological experiments. S.T. and A. M. designed chemical experiments. S.T., A. M., K.Y, P.S., and Y.O. performed chemical experiments. L.Z. and Y.K. performed the experiments.

Notes

The authors declare no competing financial interest.

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