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SYNTHESIS OF NOVEL DIKETOPIPERAZINE DERIVATIVE AND OBSEVATION OF SELF-ASSEMBLED STRUCTURE

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Abstract – An *N*-monomethylated unsymmetrical diketopiperazine was synthesized from D-*p*-hydroxyphenylglycine and sarcosine, and condensed with *trans*-1,4-cyclohexanedicarboxylic acid to obtain the ester having diketopiperazine moieties at the both termini. Atomic force microscope measurement indicated that the ester formed a supramolecular structure aligned in a circular pattern based on hydrogen bonding between the amide groups of the diketopiperazine moieties.

INTRODUCTION

Diketopiperazine (DKP), the smallest cyclic peptide, has two s-*cis* amide groups that form tandem hydrogen-bonding strands.[1–5] This nature enables DKPs to take regulated higher order structures such as liquid crystalline[6] and microcapsules[7] by appropriately modifying the side chains. Phenylalanine-, aspartic and glutamic acid-based DKPs serve as oil and ionic liquid gelators[8–10] due to the strong ability to form intermolecular hydrogen bonding even in the presence of large amount of solvents. In the field of polymer chemistry, several attempts have been made to utilize DKPs as components of polymers that largely interact with polymeric and monomeric compounds. We have recently reported the polycondensation of acidic amino acid DKPs with various diamines and dibromoxylenes to obtain polyamides and polyesters.[11] We have also performed the acyclic diene metathesis polycondensation of glutamic acid DKP ω-alkenyl esters with ruthenium catalysts.[12] The formed polymers are associated in the solid and solution states based on hydrogen bonding between the DKP moieties.

DKP derivatives are commonly poorly soluble in solvents due to the lack of flexibility of the DKP ring, which is caused by the confinement of the amide groups to the ring, as well as self-assembling based on hydrogen bonding between the amide groups. We have confirmed that DKPs having long alkyl groups aggregate based on hydrophobic interaction between the alkyl groups together with hydrogen bonding.[13] In this manuscript, we synthesize a novel *trans*-1,4-cyclohexanedicarboxylate **1** substituted with an unsymmetrical DKP consisting of D-*p*-hydroxyphenylglycine and sarcosine (Figure 1), and observe aggregates at the solid state by atomic force microscope (AFM) measurement.

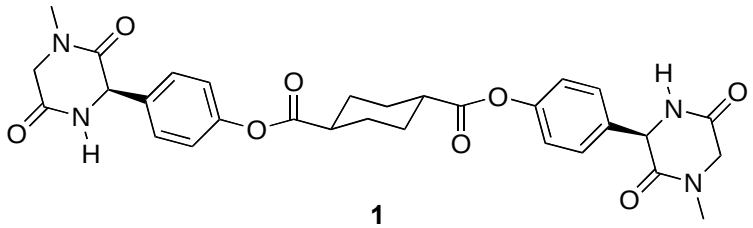

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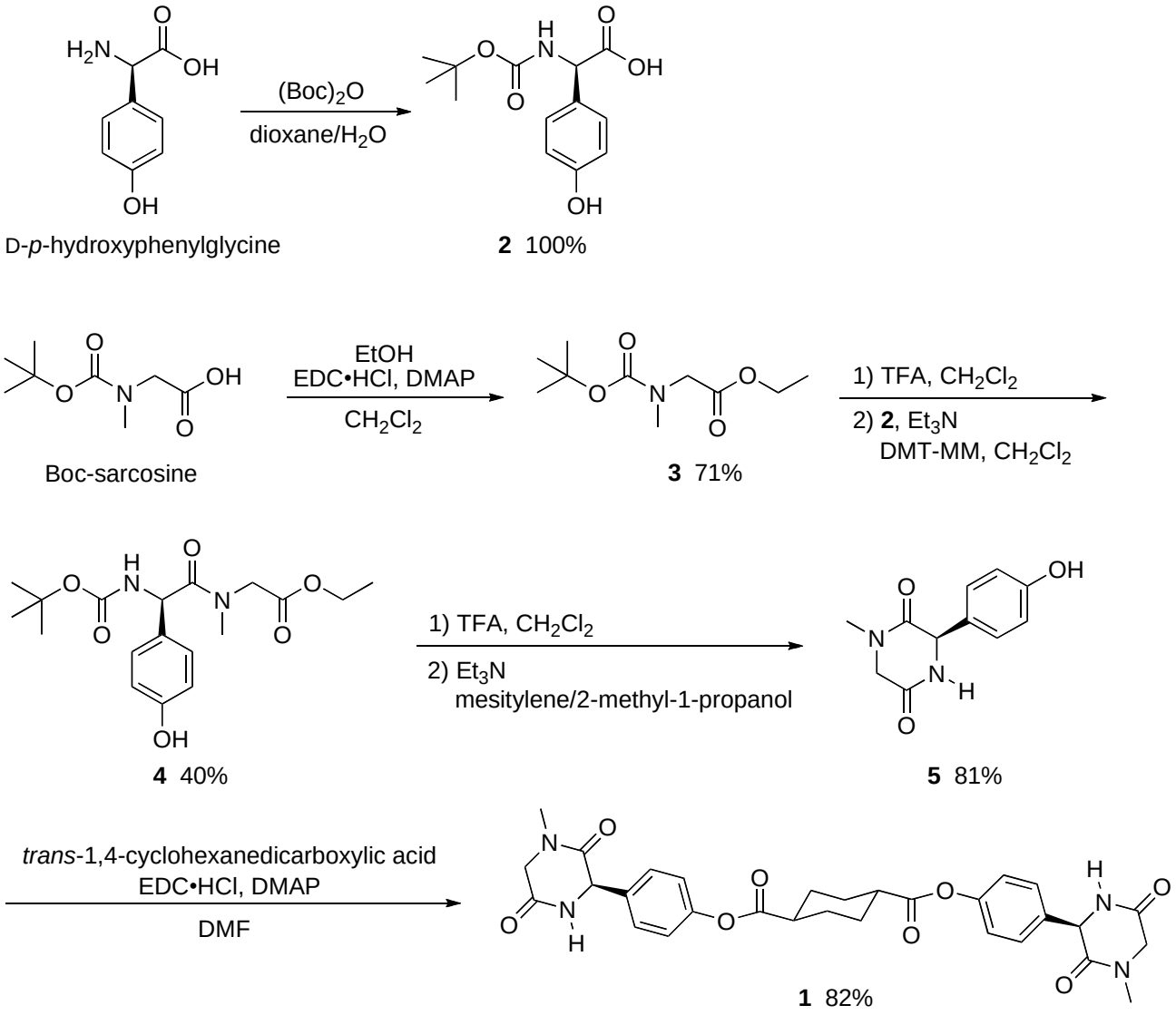
Figure 1. Structure of compound **1**

RESULTS AND DISCUSSION

We had designed DKP–linker–DKP type compounds having DKP moieties at the both termini to construct supramolecular structures. We selected D-*p*-hydroxyphenylglycine as one component of DKP, because it is commercially available and has hydroxy group, which is connectable to a linker part. Serine and threonine also have hydroxy group and satisfy this demand, but it is considered that phenylene spacer between the hydroxy group and chiral center of D-*p*-hydroxyphenylglycine is rigid compared with methylene and methine spacers of serine and threonine. Consequently, use of D-*p*-hydroxyphenylglycine seems to be more effective for constructing regulated structures. We first tried to synthesize unsymmetrical DKPs from D-*p*-hydroxyphenylglycine with several amino acids such as glycine, alanine, and leucine, but the formed compounds were poorly soluble in organic solvents, and it was difficult to purify and isolate them. We resolved the solubility problem by employing sarcosine (*N*-methylglycine) as another component. As the result, we could obtain a highly pure solvent-soluble diketopiperazine **5** (Scheme 1). *N*-Monomethylation was truly effective to enhance the solubility. We then tried to synthesize DKP–linker–DKP compounds with various linkers including xylylene, phenyleneethynylene, phenylenesilylene, and ethylenesilylene, but could not obtain the target compounds with sufficient purities. Compound **1** having *trans*-1,4-cyclohexanedicarboxylate linker could be synthesized in the present study.

Scheme 1 illustrates the synthetic route for **1**. The amino group of D-*p*-hydroxyphenylglycine was protected with Boc to obtain **2**, and it was submitted to condensation with sarcosine ethyl ester, which was prepared by ethyl esterification of *N*-(*tert*-butoxycarbonyl)sarcosine (Boc-sarcosine) followed by Boc

cleavage with trifluoroacetic acid (TFA). DMT-MM {4-[4,6-dimethoxy-1,3,5-triazin-2-yl]-4-methylmorpholinium chloride} was used as a condensation agent to avoid ester formation between the carboxy and hydroxy groups of **2**. DMT-MM can selectively react with amine to form amide with carboxylic acid in the presence of alcohol.[14] Then the terminal Boc group of formed dipeptide **4** was removed with TFA, and the resulting TFA salt was heated in the presence of triethylamine in mesitylene/2-methyl-1-propanol. The intramolecular ester-amide exchange releasing ethanol satisfactorily proceeded to give diketopiperazine **5** in a high yield (81%). Diketopiperazine **5** was obtained as precipitate during the reaction. The purity of **5** was high enough. It is considered that the precursor *N*-nonprotected linear dipeptide was completely soluble in the solvent, while **5** was slightly soluble in mesitylene/2-methyl-1-propanol mixed solvent. Diketopiperazine **5** was soluble in DMF and DMSO. Subsequently, **1** was synthesized by the condensation of *trans*-1,4-cyclohexanedicarboxylic acid with two equivalents of **5** using EDC•HCl {1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride} as a condensation agent in the presence of DMAP {4-(dimethylamino)pyridine}.



Scheme 1. Synthetic route for compound **1**

Compound **1** was insoluble in common organic solvents except DMSO, although one amide N–H group was methylated for enhancing the solubility. We measured the circular dichroism spectra of a solution of **1** in DMSO to examine the possibility of formation of a chiral supramolecular structure, but could not observe a Cotton effect at all. DMSO possibly breaks hydrogen bonding between the amide groups of **1** molecules to prevent them from association. We then examined the morphology of **1** in the solid state by AFM. Figure 2 shows the AFM image of a silicon substrate coated with a solution of **1** in DMSO. It exhibited circular objects together with a linear ribbon-like structure. Judging from the size (diameter: close to 10 nm, height: close to 1 nm)[15] of the circular structure, it seems that molecules of **1** aggregated on a silicon surface during the process of DMSO evaporation. The linear structure looks like a self-assembly of the circular objects.

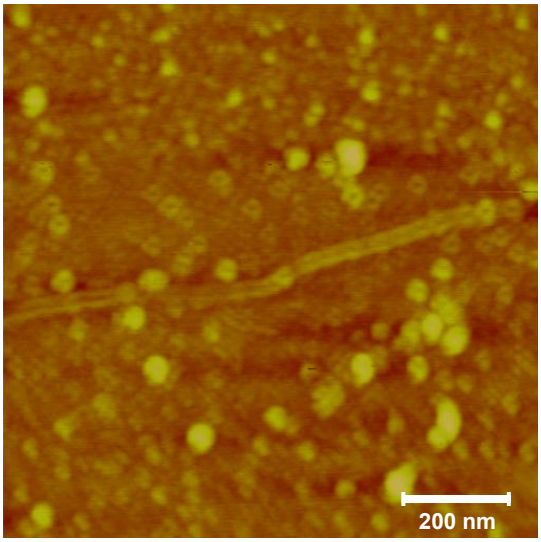


Figure 2. AFM image of **1** coated on a silicon wafer from a 0.2 w/v% solution in DMSO.

Considering the spread symmetrical structure of **1** having DKP moieties at the both termini, it is likely that **1** forms intermolecular hydrogen bonding between the DKP moieties to construct cyclic supramolecuels observed in the AFM. To elucidate this assumption, we modeled supramolecular structures consisting of 2–16 units of **1**, wherein hydrogen bonding exists between the DKP moieties. Figure 3 plots the potential energy per unit and diameter of the ring structure calculated with the molecular mechanics method using the MMFF94 force field.[16] We postulated that the dimer would aggregate in a linear fashion and that the trimer and the tetramer would aggregate based on a single-strand of hydrogen bonding between DKP moieties to form the ring structures. Oligomers consisting of five and more units would aggregate based on double-strands of hydrogen bonding to form cyclic structures. The energy per one unit gradually decreased with the increment of unit number (n), and became saturated around $n = 5$. The diameter of the ring increased in proportion to n. For example, the diameter of 10.4

nm corresponded with a ring consisting of 14 molecules of **1** as illustrated in Figure 4, whose height was calculated to be 1.1 nm. The value was coincident with the value (1.0 nm) observed by AFM. Consequently, these data suggest that the AFM-observed ring structure with a diameter of 10.4 nm is cyclic 14-mer of **1** aggregated by intermolecular hydrogen bonding between the terminal DKP moieties. It seems that cyclic oligomers larger and smaller than 14-mer also exist judging from the distribution of ring size observed in Figure 2 and the energy diagram in Figure 3.

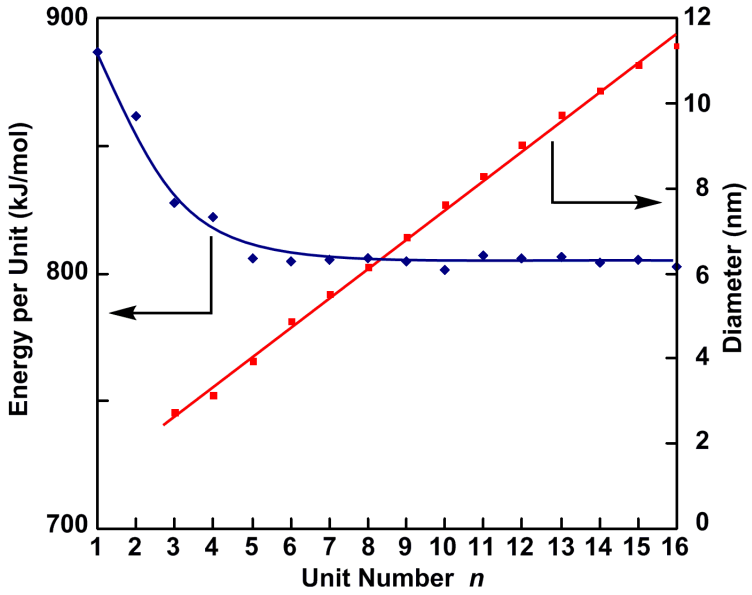


Figure 3. Unit number vs. energy per unit and diameter of cyclic supramolecules of **1**.

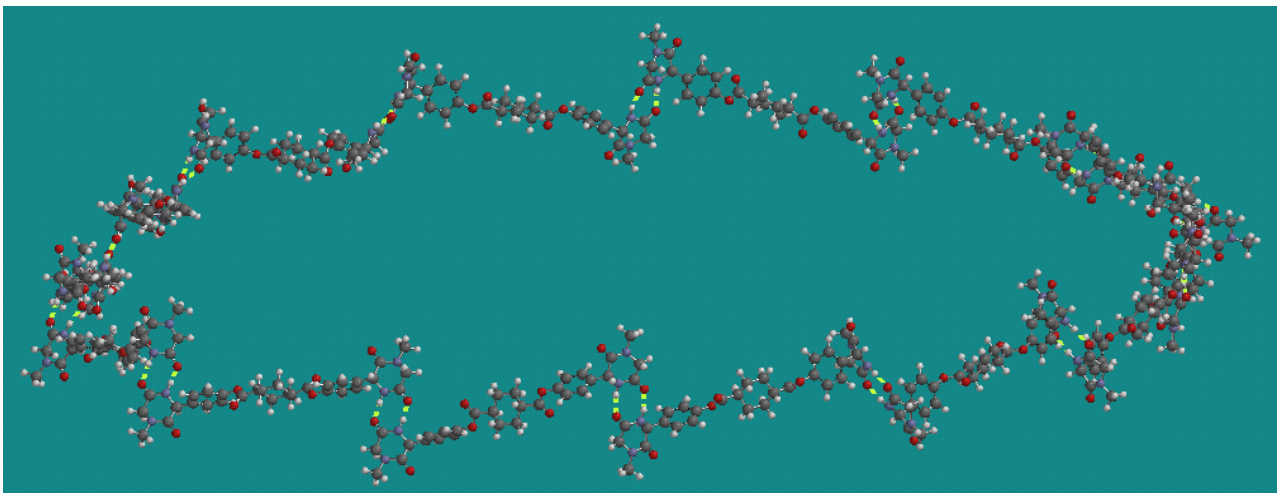


Figure 4. Cyclic 14-mer of **1** optimized by the molecular mechanics calculation. Green dotted lines represent hydrogen bonds between the DKP amide moieties.

We have demonstrated the synthesis of novel compound **1** having two DKPs consisting of D-*p*-hydroxyphenylglycine and sarcosine. AFM measurement and molecular mechanics calculations

suggested that **1** formed cyclic supramolecular structures based on intermolecular hydrogen bonding between the terminal DKP moieties on silicon surface. It is expected that further molecular design of a linker tethering two DKPs leads to variation of supramolecular structures.

EXPERIMENTAL

Measurements. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL EX-400 spectrometer. Melting points (mp) were measured on a Yanaco micro melting point apparatus. Specific rotations ($[\alpha]_D$) were measured on a JASCO DIP-100 digital polarimeter with a sodium lamp as a light source. Elemental analysis was done at the Microanalytical Center of Kyoto University. Mass spectra were measured on a JEOL JMS-HX110A mass spectrometer. A sample for AFM measurement was prepared by dropping a solution of **1** in DMSO (0.2 w/v%) on a silicon substrate, followed by washing with DMSO and drying *in vacuo*. Prior to preparation of the sample, the silicon substrate was treated with a fresh piranha solution at 100 °C for 1 h to form clean silicon oxide surface, followed by extensive rinsing with deionized water, and then dried under nitrogen flow. A Nanoscope III (Digital Instruments, Inc.) was used for tapping-mode AFM to observe phase and height images.

Materials. All the reagents in monomer synthesis were used as purchased without purification. DMF was distilled over calcium hydride.

Synthesis of 1,4-*trans*-bis[cyclo(D-*p*-phenylglycinylsarcosine)]cyclohexane dicarboxylate (1). ***N*-(*tert*-Butoxycarbonyl)-D-*p*-hydroxyphenylglycine (2)** Triethylamine (11 mL, 79.0 mmol) was added to a mixture of D-*p*-hydroxyphenylglycine (8.84 g, 52.9 mmol) and di-*tert*-butyl dicarbonate [$(Boc)_2O$, 11.5 g, 52.9 mmol] in dioxane/H_2O (70 mL/70 mL) at 0 °C, and the reaction mixture was stirred at rt overnight. H_2O (100 mL) was added to the resulting mixture, and then it was washed with EtOAc. The aqueous layer was acidified with 2 M HCl to pH 2, and then extracted with EtOAc. The organic layer was dried with anhydrous $MgSO_4$ and concentrated on a rotary evaporator to obtain **2** as a yellow solid quantitatively. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.40 [s, 9H, $-OC(CH_3)_3$], 5.02 [s, 1H, >NCH<], 5.19 [s, 1H, –ArOH], 5.73 [s, 1H, –NH–], 6.70–7.16 [m, 4H, Ar], 8.28 [br, 1H, –OH].

***N*-(*tert*-Butoxycarbonyl)sarcosine ethyl ester (3)** EDC•HCl（19.2 g, 100 mmol), DMAP (1.22 g, 9.99 mmol), and EtOH (10 mL, 171 mmol) were added to a solution of *N*-(*tert*-butoxycarbonyl)sarcosine (18.9 g, 99.9 mmol) in CH_2Cl_2 (500 mL) at 0 °C, and then the resulting mixture was stirred at rt overnight. Then, CH_2Cl_2 was distilled off using a rotary evaporator, and the residue was dissolved in EtOAc. It was washed with 0.5 M HCl, saturated $NaHCO_3$ aq., and saturated NaCl aq., and then dried over anhydrous $MgSO_4$. EtOAc was evaporated off to obtain **3** as a colorless liquid in 71% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27, 1.29 [s, 3H, $-CH_2CH_3$], 1.43, 1.47 [s, 9H, $-OC(CH_3)_3$], 2.92, 2.94 [s, 3H, $>NCH_3$], 3.88, 3.97 [s, 2H, $>NCH_2-$], 4.20 [s, 2H, $-OCH_2-$].

***N*-(*tert*-Butoxycarbonyl)-D-*p*-hydroxyphenylglycinylsarcosine ethyl ester (4)** Trifluoacetic acid

(TFA, 15.0 mL, 202 mmol) was added to a solution of **3** (8.43 g, 38.8mmol) in CH_2Cl_2 (100 mL) at 0 °C, and the reaction mixture was stirred at rt overnight. After confirming the complete consumption of **3** by TLC, CH_2Cl_2 and TFA were distilled off *in vacuo*. The residual viscous liquid was dissolved in EtOAc/CH_2Cl_2 (200 mL/100 mL). Triethylamine (17 mL, 122 mmol), DMT-MM (Tokuyama Co.), 12.6 g, 38.8 mmol), and **2** (13.4 g, 50.1 mmol) were added to the solution at 0 °C, and the resulting mixture was stirred at rt overnight. The resulting mixture was concentrated on a rotary evaporator, and then the residual viscous liquid was dissolved in CH_2Cl_2 (150 mL). It was washed with 0.5 M HCl, saturated $NaHCO_3$ aq., and saturated NaCl aq., and then dried over anhydrous $MgSO_4$. CH_2Cl_2 was evaporated off, and the residual mass was purified by recrystallization from hexane/EtOAc (1/2, volume ratio) to obtain **4** as a colorless solid in 40%. Mp 155–161 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24 [t, J = 6.8 Hz, 3H, –CH_2CH_3], 1.41 [s, 9H, –$OC(CH_3)_3$], 2.93, 3.01 [s, 3H, >NCH_3], 3.88, 4.34 [d, J = 8.8 Hz, 2H, >NCH_2–], 5.54, 5.92 [d, J = 4.0 Hz, 1H, >NCH<], 7.01 [s, 1H, –ArOH], 7.14 [d, J = 4.4 Hz, 1H, –NH–], 6.74–7.23 [m, 4H, Ar]. ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.07 [CH_3CH_2–], 28.32 [$(CH_3)_3C$–], 36.29 [>NCH_3], 50.09 [>NCH_2–], 54.67 [>NCH<], 61.38 [CH_3CH_2–], 80.01 [$(CH_3)_3C$–], 115.86, 128.35, 129.19, 155.25 [Ar], 156.35 [–OCONH–], 168.87[>CHCON<], 171.32[–CH_2COO–]. Anal. Calcd for $C_{18}H_{26}N_2O_6$: C, 59.00; H, 7.15; N, 7.65. Found: C, 58.87; H, 7.12; N, 7.52.

***cyclo*(D-*p*-Hydroxyphenylglycinylsarcosine) (5)** TFA (11 mL, 148 mmol) was added to a solution of **4** (5.54 g, 15.1mmol) in CH_2Cl_2 (100 mL) at 0 °C, and the reaction mixture was stirred at room temperature overnight. After confirming the complete consumption of **4** by TLC, CH_2Cl_2 and TFA were distilled off *in vacuo*. The residual viscous liquid was dissolved in mesitylene/2-methyl-1-propanol (2/1, volume ratio), and then triethylamine (5.00 mL, 35.9 mmol) was added to the resulting solution. The resulting mixture was heated to 110 °C and stirred for 11 h. After standing to cool to rt, a white solid precipitated was separated by filtration to obtain **5** as a colorless solid in 81%. Mp (decomposed at 250–257 °C). $[\alpha]_D$ −41.4° (*c* 0.10 g/dL, MeOH at rt). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.80 [s, 3H, >NCH_3], 3.87, 4.11 [d, J = 8.8 Hz, 2H, >NCH_2–], 4.79 [s, 1H, >NCH<], 6.72–7.11 [m, 4H, Ar] , 8.59 [s, 1H, –NH–] 9.48 [s, 1H, –ArOH]. ^{13}C NMR (100 MHz, DMSO-d_6): δ 33.16 [>NCH_3], 50.96 [>NCH_2–], 58.10 [>NCH<], 115.18, 127.97, 129.47, 157.06 [Ar], 164.77[–CH_2CO–], 164.96[>NCO–]. Anal. Calcd for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 59.80; H, 5.53; N, 12.68. HRMS (M^+). Calcd for $C_{11}H_{12}O_3N_2$ (m/z) 220.0848. Found: 220.0847.

1 EDC•HCl (959 mg, 4.99 mmol), 4-(dimethylamino)pyridine (DMAP, 61.0 mg, 0.499 mmol), *trans*-1, 4-cyclohexanedicarboxylic acid (431 mg, 2.50 mmol) were added to a solution of **5** (1.10 g, 4.99 mmol) in DMF (90 mL) at 0 °C and the resulting mixture was stirred at rt overnight. The precipitate formed was separated by filtration and washed with H_2O and MeOH to obtain **1** as a colorless solid in 82%. Mp (decomposed at 225–227 °C). $[\alpha]_D$ –51° (*c* 0.10 g/dL in DMSO at rt). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.57–2.64 [m, 8H, –C_6H_{10}–], 2.82 [s, 6H, >NCH_3], 4.04 [d, J = 9.3 Hz, 4H, >NCH_2–], 4.99

[s, 2H, >NCH<], 7.11–7.38 [m, 8H, Ar] , 8.71 [s, 2H, –NH–]. ^{13}C NMR (100 MHz, DMSO-d_6): δ 27.37 [>CH(C_2H_4)$_2$CH<], 33.24 [>NCH_3], 41.43 [>CH(C_2H_4)$_2$$C$H<], 50.95 [>$NCH_2$–], 58.06 [>NCH<], 121.86, 128.20, 136.73, 150.17 [Ar], 164.28[–CH_2CO–], 164.97[>NCO–], 173.61 [–OCO–]. IR (cm^{-1}, KBr): 3570 (NH), 3425 (NH), 3232 (NH), 2923 (CH), 2864 (CH), 1728 (C=O), 1673 (NHCO), 1510, 1475, 1451, 1321, 1167, 1148, 1013, 804. HRMS (M^+). Calcd for $C_{30}H_{32}O_8N_4$ (m/z) 577.2298. Found: 577.2296.

Molecular mechanics calculation. The molecular mechanics calculation was carried out with Wavefunction Inc., Spartan '06 Windows, using the MMFF94 forcefield.

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REFERENCES AND NOTES

1. Y. Du, C. J. Creighton, B. A. Tounge, and A. B. Reitz, *Org. Lett.*, 2004, **6**, 309.
2. K. A. Lyssenko, D. A. Lenev, and R. G. Kostyanovsky, *Tetrahedron*, 2002, **58**, 8525.
3. S. Palacin, D. N. Chin, E. E. Simanek, J. C. MacDonald, G. M. Whitesides, M. T. McBride, and G. T. R. Palmore, *J. Am. Chem. Soc.*, 1997, **119**, 11807.
4. D. N. Chin, T. R. Palmore, and G. M. Whitesides, *J. Am. Chem. Soc.*, 1999, **121**, 2115.
5. B. Jagadosh, M. D. Carducci, C. Bosshard, P. Günter, J. I. Margolis, L. J. Williams, and E. A. Mash, *Cryst. Growth Des.*, 2003, **3**, 811.
6. R. A. Kloster, M. D. Carducci, and E. A. Mash, *Org. Lett.*, 2003, **5**, 3683.
7. R. J. Bergeron, O. Phanstiel IV, G. W. Yao, S. Milstein, and W. R. Weimar, *J. Am. Chem. Soc.*, 1994, **116**, 8479.
8. Y. Yang, M. Suzuki, M. Kimura, H. Shirai, and K. Hanabusa, *Chem. Commun.*, 2004, 1332.
9. K. Hanabusa, M. Matsumoto, M. Kimura, A. Kakehi, and H. Shirai, *J. Colloid Interface Sci.*, 2000, **224**, 231.
10. K. Hanabusa, H. Fukui, M. Suzuki, and H. Shirai, *Langmuir*, 2005, **21**, 10383.
11. K. Terada, F. Sanda, and T. Masuda, *J. Macromol. Sci., Part A: Pure Appl. Chem.*, 2007, **44**, 789.
12. K. Terada, E. B. Berda, K. B. Wagener, F. Sanda, and T. Masuda, *Macromolecules*, 2008, **41**, 6041.
13. Y. Ohta, K. Terada, T. Masuda, and F. Sanda, *Heterocycles*, 2009, **78**, 1477.
14. M. Kunishima, C. Kawachi, K. Hioki, R. Terao, and S. Tani, *Tetrahedron*, 2001, **57**, 1551.
15. See, Supporting Information.
16. T. A. Halgren, *J. Comput. Chem.*, 1996, **17**, 490.