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Peptidehelicenes in solution and gel: chiral discrimination through interactions between two types of helixes

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ABSTRACT

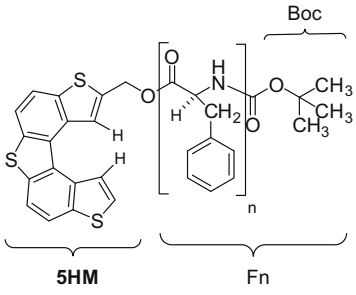
Helical [5]thiaheterohelicene **5HM**, which rapidly interconverts between *P* and *M* enantiomers in solution, was connected to helical L-phenylalanine oligomers with an ester linkage to give peptidehelicenes (**5F*n***, where *n*: number of bonded phenylalanines). The characteristics of **5F4** and **5F5** with two types of helixes in a molecule were investigated, particularly in comparison with those of **5F1**–**5F3** with an incomplete coil of a peptide moiety. L-Phenylalanine peptide chains induced a shift in the equilibrium between the *P* and *M* helixes of **5HM** toward the *P* side for all the **5F*n***s examined. The enantiomeric excess (ee) of the *P* form increased with a decrease in temperature, together with an elongation of the peptide chains. **5F4** and **5F5** in hot solutions of some solvents formed a gel at room temperature, whereas **5F1**–**5F3** showed no such behavior. In this gel, the stable helical form of the **5HM** moiety in **5F4** and **5F5** was observed to be the *M* form in contrast to that in their solutions.

1. Introduction

When a helical structure is formed in a molecule or a system, it is interesting to learn how the sense of a helix is determined, in connection with the formation of natural helical substances. Along this line, much research has been reported in recent years. For various molecules and substances, such as artificial polymers,[1,2] molecular aggregates,[3,4] and supramolecules,[5,6] a major group of a large achiral portion is controlled by a minor group of a small chiral portion, to form a large chiral environment (Sergeants and Soldiers effects). Furthermore, when a slight excess of either enantiomer is produced in a racemic system, the chirality of the entire system is changed to the same sense as that of the enantiomer (Majority Rules[7]). In these phenomena, local chirality spreads out over its circumference through the interaction between chiral molecules and achiral or other chiral molecules, fixing the whole chirality sense in a system. These types of interaction concerning chirality control have found several practical applications such as to the synthesis of liquid crystal materials,[8] the optical active films,[9] the fabrication of electronic biodevices,[10] the helical nanotubes,[11] and the development of new methods for asymmetric syntheses[12] using small amounts of enantiomeric catalysts.

Herein, we describe the synthesis and properties of molecules with two types of helixes; a peptide helix and a flexible helicene ([5]thiaheterohelicene, **5HM**). The interaction between these two helixes has aroused our interest, particularly, as regard to the difference between the interactions of **5HM** with tripeptides having an incomplete helix and with tetrapeptides having a complete helix. The peptides used here consist of L-phenylalanine (Phe, F) oligomers with its N-terminal being protected by a *tert*-butoxycarbonyl (Boc) group. A peptide chain produces a complete α-helix coil with approximately 3.6 amino acid residues. We tentatively call the present molecules 'peptidehelicenes' in which a Phe oligomer is bonded to a **5HM** moiety with an ester linkage. These peptidehelicenes are indicated as **5F*n*** (*n* = 1–5), where *n* is the number of Phes as shown in Scheme 1. Thus, **5F4** and **5F5** possess both one complete peptide helix and a **5HM** helix in each molecule.

5HM, belonging to the helicene family, has a helical shape arising from the steric repulsion between its terminal hydrogen



Scheme 1. Structures of **5F*n*** (*n* = 1–5).

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atoms.[13] However, such repulsion is too weak in solution to maintain its helical skeleton, causing a rapid inversion of its helix. Therefore, **5HM** in solution shows an equilibrium between right-handed (*P*) and left-handed (*M*) configurations without inducing any circular dichroism (CD) absorption. Once **5HM** is placed in chiral environments, such as micelles,[14] membranes,[15] and proteins,[16] the equilibrium shifts to either side of the *P* or *M* enantiomer, inducing CD absorption. The direction and extent of the equilibrium shift can be estimated from the sign and intensity of Cotton effects in CD absorptions, respectively. The induced CD based on **5HM** is considerably intense and appears in long-wavelength regions, a characteristic of its widely conjugated chromophore, where the absorption seldom overlaps with those of peptides.

2. Results and discussion

2.1. Synthesis of 5F*n*

The synthetic routes of **5F*n*** are summarized in Scheme 2. The key step in the synthesis of **5F1** and **5F2** was the ester formation between **5HM** and Boc-(Phe)$_n$ (n = 1, 2) by the actions of 4-dimethylaminopyridine (DMAP)[17] and dicyclohexylcarbodiimide (DCC). In the course of the synthesis of **5F3**, **5F4**, and **5F5**, it was important to prevent the racemization of the stereogenic carbons. Thus, Boc was first removed from **5F*n*** (n = 1, 2) to be transformed to hydrogen chloride salts which were combined with Boc-(Phe)$_2$ or Boc-(Phe)$_3$ in the presence of DCC.

2.2. Configuration of 5HM moiety in L-5F1 and D-5F1

Since a mutual, rapid conversion between *P* and *M* enantiomers takes place in solution, **5HM** in principle induces no CD absorption. However, when an L-Phe molecule is linked to **5HM**, a pair of diastereoisomers, *P*-**5F1** and *M*-**5F1**, becomes feasible; the two compounds have different stabilities. This may cause a shift in the equilibrium of the **5HM** moiety to the more stable side, that is, to either the *P* or *M* side; therefore CD absorption may be induced depending on the excess of *P* or *M* form. The attachment of L-phenylalanine to **5HM** (**5-L-F1**) induced CD absorptions, though weak, in THF (Fig. 1), suggesting a small shift in the equilibrium. It is interesting that the spectra of both **D-5F1** and **L-5F1** are almost complete mirror images (Fig. 1). This implies that both compounds exist as enantiomers in solution. The absorption bands of members of the helicene family have been assigned to the α, β and p transitions by Clar.[18] The negative Cotton effect of the α band along with the positive Cotton effects of the p and β bands indicates that the *P* form dominates over its antipode, as determined from the comparison of those Cotton effects with the Cotton effects of *P* [7]heterohelicene with no helix inversion.[19] Therefore, it is concluded that **L-5F1** preferentially induces the *P* form, and that **D-5F1** reversely induces the *M* form. In other words, L-Phe in **5F1** promotes the helicity of **5HM** with a rapid interconversion even at −20 °C[20] to the right-handed one at room temperature, and vice versa.

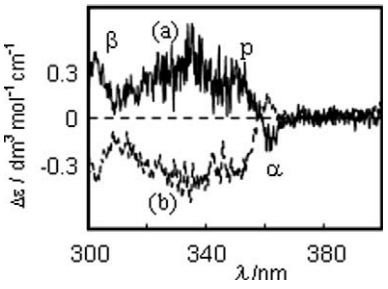
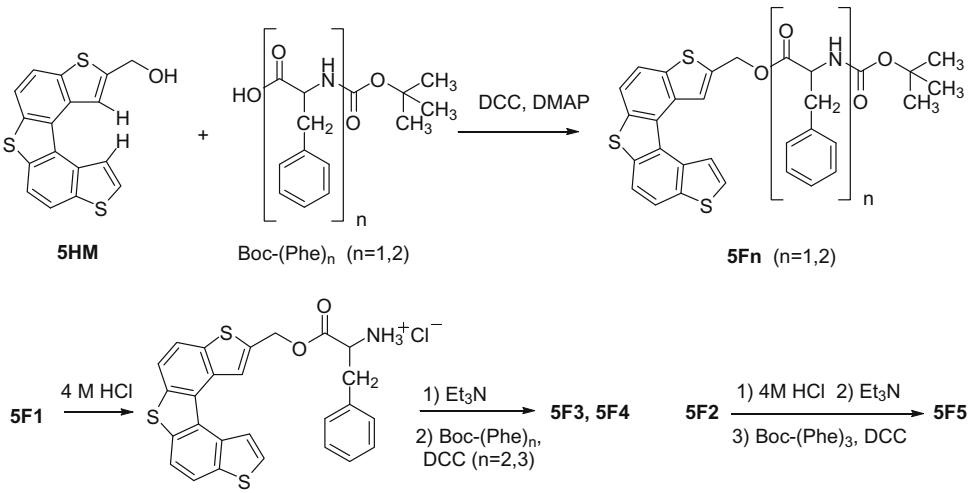


Figure 1. CD spectra of **5-L-F1** (a) and **5-D-F1** (b) in THF at rt.

2.3. Effect of peptide length of 5F*n* on configuration

In **5F1**, the predominant chirality of the helicene moiety was determined by the chirality of Phe used in **5F1**: L-Phe induced the *P*-configuration and D-Phe induced the *M*-configuration. Next, we examined how the extent of configuration of the helicene moiety is affected by an increase in the length of the peptide chain of **5F*n*** (n = 2–5). In particular, we were interested in **5F4** and **5F5** having a coil longer than one helix. Figure 2 shows the CD spectra of **5F1** to **5F5** in THF at −100 °C. The intensity of each CD absorption increased markedly with an increase in the length of peptides. All the spectra demonstrated similar shapes, with the signs of Cotton effects being the same. This indicates that the helicity of the helicene moiety in **5F*n*** is controlled by the chirality of Phe, with similar wavelengths of peaks and troughs, regardless of the length of peptides.



Scheme 2. Synthesis of **5F*n*** (n = 1–5).

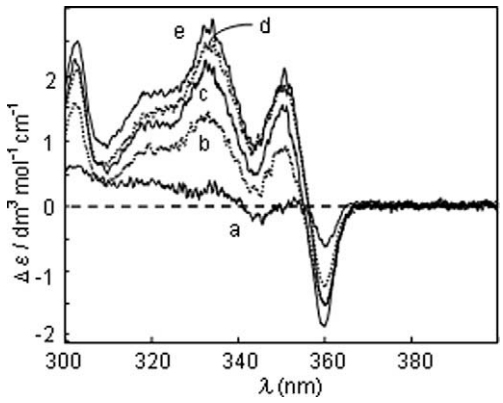


Figure 2. CD spectra of **5Fn** (n = 1–5) in THF at −100 °C. (a) **5F1**, (b) **5F2**, (c) **5F3**, (d) **5F4**, (e) **5F5**. [**5F1**] = 5.2×10^{-5} M, [**5F2**] = 5.7×10^{-5} M, [**5F3**] = 5.2×10^{-5} M, [**5F4**] = 5.5×10^{-5} M, [**5F5**] = 5.2×10^{-5} M.

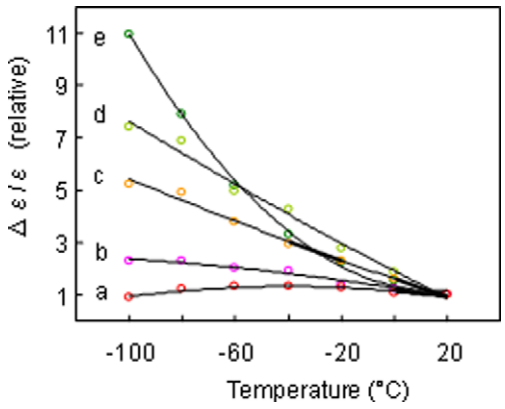


Figure 4. Relative intensities of $\Delta\varepsilon/\varepsilon$ of **5Fn**. (a) **5F1**, (b) **5F2**, (c) **5F3**, (d) **5F4**, and (e) **5F5**, monitored at approximately 350 nm.

2.4. Effect of temperature on configuration

Figure 3 shows the temperature effects on the CD and UV spectra of **5F4** at 20 to −100 °C in THF. With a decrease in temperature, CD intensity markedly increased (Fig. 3A), indicating the equilibrium shift to the *P*-configuration side. On the other hand, the absorption bands of the UV spectra became sharp with decreasing temperature, because of a change of electron populations on the vibrational levels in solution. The contributions of the equilibrium shift to the increase in CD intensity with decreasing temperature can be estimated by alterations of anisotropy factors ($\Delta\varepsilon/\varepsilon$), where $\Delta\varepsilon$ ($=\varepsilon_l - \varepsilon_d$) is the circular dichroism and ε is the molecular absorptivity in UV absorption.[21] The temperature dependence of $\Delta\varepsilon/\varepsilon$ at λ_{max} of approximately 350 nm (p band) is plotted in Fig. 4, in which the values in the ordinate are normalized in such a way that $\Delta\varepsilon/\varepsilon = 1$ at 20 °C. Generally, for the system in which the structure of a stereogenic center does not change with temperature, such normalized $\Delta\varepsilon/\varepsilon$ values as mentioned above show small fluctuations from approximately 1 with varying temperature. However, the present case seems very different. $\Delta\varepsilon/\varepsilon$ increased rapidly with decreasing temperature and increasing length of the peptide chains in **5Fn**. The variations of $\Delta\varepsilon/\varepsilon$ with temperature were relatively small for **5F1** and **5F2**, but relatively large for **5F3** with almost one helical coil of peptide, and for **5F4** and **5F5** with peptides longer than one helical coil. These imply that the lower the temperature and the longer the peptide chain, the more enhanced the extent of configuration of the helicene moiety. Namely, the reduced temperature and the long peptide chain effectively prevent the inversion from the *P* form to the *M* form of the helicene moiety, thereby inducing intense CD absorption.

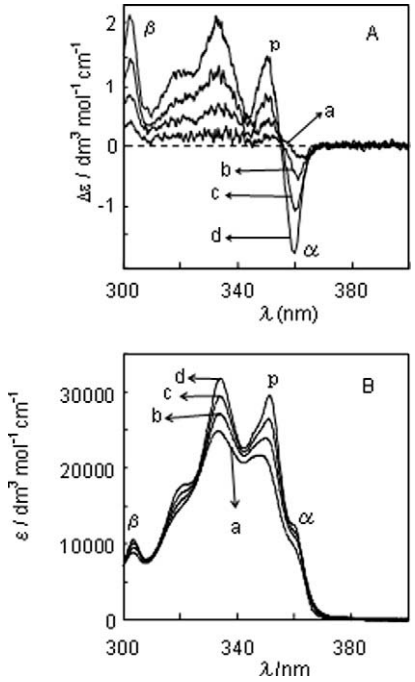


Figure 3. Effect of temperature on CD spectra (A) and UV–vis spectra (B) of **5F4** in THF. [**5F4**] = 5.4×10^{-5} M. (a) 20, (b) −20, (c) −60, (d) −100 °C.

2.5. Gel formation and chiral discrimination

When a hot solution of **5F4** in CH_3CN (ca. 2.2×10^{-3} M, 0.22 w/v%) was slowly cooled to purify it by recrystallization, gelation took place suddenly to give a transparent gel (Fig. 5). The gel was stable at least over one day at room temperature. Among several solvents examined, only CH_3CN solution of **5F4** gave such a gel. In order to measure the UV and CD spectra of this gel, a hot CH_3CN solution was put in a combination cell with a 0.2 mm path length, forming a thin gel film. Figure 6 shows the CD and UV spectra of the **5F4** gel at room temperature together with the spectra of the **5F4** solution at −100 °C. The UV spectrum of the gel became broader than that of the solution, because of lower molecular mobility. On the other hand, the CD spectra revealed that the enhanced intensity of the gel was over 8 times larger than that in the solution. Of particular interest was the reversal of the signs of Cotton effects. These imply

Figure 5. Gel of **5F4** in a sample vial. [**5F4**] = 2.2×10^{-3} M in CH_3CN at rt.

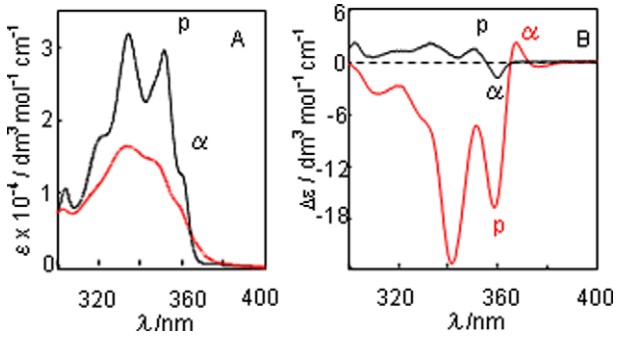


Figure 6. UV–vis (A) and CD (B) spectra of **5F4**. Solution spectra (black) were measured in THF at −100 °C and [**5F4**] = 5.4×10^{-5} M. CH_3CN gel spectra (red) were measured at rt and [**5F4**] = 2.2×10^{-3} M.

that (1) the equilibrium between the *P* and *M* diastereomers of the helicene moiety shifts by gelation, the *M*-configuration being overwhelmingly predominant over the *P* form and that (2) the environments surrounding the helicene moiety of **5F4** are different between the solution and the gel in terms of chiral discrimination.

As for the other **5F*n*** compounds, **5F1**, **5F2**, and **5F3** with relatively shorter peptide chains showed no gelation. For **5F5**, gelation similar to that observed in **5F4** took place. Therefore, it is understood that such gelation requires a relatively longer peptide chain. **5F5** had a poor solubility in CH_3CN, that is, it does not set as a gel in this solvent. However, it showed an ability to form a gel in various kinds of solvents, such as $CHCl_3$, CH_2Cl_2, $CHCl_3/CH_3OH$, $CHCl_3/C_2H_5OH$, $CHCl_3/C_6H_6$, THF/C_2H_5OH, and 1,4-dioxane/C_6H_{14} at concentrations of 1 – 3 × 10^{-3} M. The CD and UV–vis spectra of **5F5** in the gel ($CHCl_3$) together with those in $CHCl_3$ solution are shown in Fig. 7. The UV–vis spectrum of **5F5** in gel (red line) showed a sharper absorption than that of **5F4** in gel, suggesting that the helicene moiety of **5F5** is in a more mobile environment. The Cotton effects and absorptional wavelengths in the CD spectrum were almost the same as those of **5F4**, in agreement with the predominant *M*-configuration of the helicene moiety. However, the intensity was much weaker than that of **5F4** for some solvent systems and almost comparable to that in solution (black line).

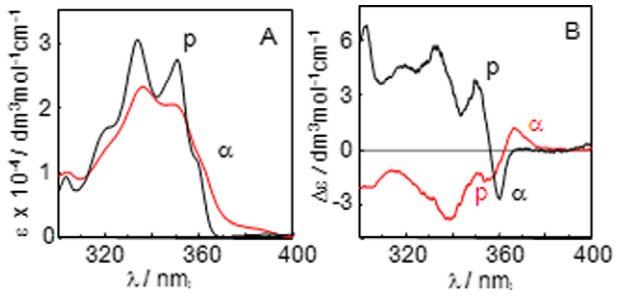


Figure 7. UV–vis (A) and CD (B) spectra of **5F5**. Solution spectra (black) were measured in THF at −100 °C and [**5F5**] = 5.6×10^{-5} M. $CHCl_3$ gel spectra (red) were measured at rt and [**5F5**] = 3.3×10^{-3} M.

In the gel, it can be tentatively presumed that the helicene moiety of **5F*n*** (*n* = 4, 5) is encapsulated within a cavity formed with hydrogen-bonding networks by solvent molecules and the peptide chains of **5F*n***. The gel of **5F5** having a longer peptide chain formed a lager cavity than the gel of **5F4**. Therefore, the helicene moiety of **5F5** is more loosely encapsulated in the cavity than that of **5F4**, resulting in a smaller equilibrium shift between the *P*- and *M*-configurations. This may be the reason why **5F5** demonstrated a weaker CD absorption than **5F4**.

2.6. Effect of temperature on gelation

When the temperature of **5F4** in CH_3CN (5.2×10^{-4} M; 10 times more concentrated than that in the case shown in Fig. 3) was gradually reduced from 20 to −10 °C, the Cotton effects of CD spectrum gradually attenuated, and at approximately 4 °C the signs of Cotton effects reversed, followed by an increase in the absolute value of intensity with further decrease in temperature. In contrast, an increase in temperature from −10 to 20 °C caused a decrease in the absolute value of intensity and no reversal of the signs was observed up to approximately 20 °C. These changes in CD absorptional intensity monitored at 340 nm are shown in Fig. 8. This figure clearly shows the hysteresis effect of solution/gel transition: the gel state at low temperatures and the solution state at high temperatures. As generally noted for gelation phenomena, the transition of a solution to a gel occurs at temperatures (approximately 4 °C) lower than that of a gel to a solution (approximately 20 °C). Although the transition temperatures of solution/gel have been measured by various methods, in the present case it is interesting that the transition temperatures were determined on the basis of the difference in chiral discrimination between the solution and the gel by CD measurement.

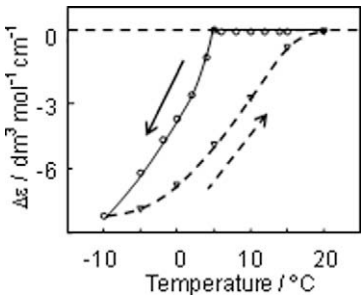


Figure 8. Hysteresis of gel/solution transition of **5F4**. [5F4] = 5.2×10^{-4} M. Intensities were monitored at 340 nm. The solid line shows Δε when temperature decreased and the broken line shows Δε when temperature increased.

3. Conclusion

Chiral discrimination was investigated for new compounds, namely, peptidehelicenes (**5F*n***), consisting of an L-phenylalanine peptide and a mobile helicene (**5HM**). The helical sense of the **5HM** moiety was determined by the chirality of the applied phenylalanine, which was commonly observed in all the **5F*n*** compounds in solution. **5F4** and **5F5**, which have a helical coil of peptide in their molecules, formed a stable organogel in some solvents. The helical sense of the **5HM** moiety in the gel state was completely opposite to that of the **5HM** moiety in the solution state at room temperature. The difference in helical sense between both states may be mainly attributable to the difference in the mode of action against **5F*n*** molecules between a solution and a gel, that is, intramolecular interaction in a solution and intermolecular interaction in a gel.

4. Experimental

4.1. General

Unless otherwise noted, all starting materials and solvents were obtained from commercial suppliers and used without further purification. *tert*-Butyloxycarbonyl-L-phenylalanine (Boc-Phe-OH), H-Phe-OMe·HCl, and Boc-D-Phe-OH were purchased from Kokusan Chemical co. (Tokyo). Boc-Phe-Phe-OH was purchased from

BACHEM co. (Torrance). 2-Hydroxymethyl[5]thiaheterohelicene (**5HM**) was prepared according to the procedures described in our previous article.[16] UV–vis spectra were performed on a JASCO V-560 spectrophotometer equipped with an ETC-505T temperature controller. CD spectra were measured on a JASCO J720W spectropolarimeter equipped with a PTC-348WI temperature controller. UV–vis and CD spectra were measured using 10, 0.5, and 0.2 mm light-path length quartz cells. Spectra at low temperatures were obtained using Oxford cryostats DN704 for UV–vis spectra and an Optistat DN for CD spectra with an ITC502 temperature controller. ^{1}H NMR was measured on a JEOL JNM-EX270FT (270.05 MHz) and a JEOL JNM-A500 (500.00 MHz). Mass spectral data were measured on a JEOL JMS-700 by means of EI and FAB techniques.

4.2. Preparation of Boc-phenylalanine methyl[5] thiaheterohelicene ester 5F1

5HM (130.6 mg, 0.4 mmol), Boc-L-Phe (123.0 mg, 0.48 mmol), and DMAP (4-dimethylaminopyridine) (12.2 mg, 0.1 mmol) were placed in a screw-capped glass vial, and 1.5 ml of anhydrous 1,4-dioxane was poured to dissolve the mixture completely. 1 mol/l DCC (dicyclohexylcarbodiimide) dioxane solution (0.4 ml, 0.4 mmol) was added to the above solution with stirring, and continuously stirred overnight. After confirming that no **5HM** remained in the solution by TLC (benzene/ethanol = 10/1), 10% acetic acid dioxane solution (0.2 ml, 0.4 mmol) was added to the solution and stirred for 15 min to convert DCC into dicyclohexyl urea (DCurea). DCurea was removed by filtration, and the filtrate was extracted with 20 ml of ethyl acetate. The ethyl acetate layer was washed with 3% HCl, water, 3% sodium hydrogen carbonate, and water, and then dried over anhydrous sodium sulfate. When the solvent was evaporated under reduced pressure, orange solid was obtained. This residue was purified by silica-gel column chromatography (dichloromethane/acetonitrile = 30/1) to give pale yellow amorphous solid of **5F1** (193.5 mg, yield 84.3%). Mp 58.3–61.5 °C and *m*/*z* 573 (calcd 573); Anal. Calcd for $C_{31}H_{27}NO_4S_3$: C, 64.90; H, 4.74; N, 2.44. Found: C, 64.71; H, 4.90; N, 2.31. ^{1}H NMR (270 Hz, $CDCl_3$): δ = 8.31 ppm (s, 1H), 8.25 (d, 1H, *J* = 5.6 Hz), 8.02 (d, 1H, *J* = 8.6 Hz), 7.94 (d, 1H, *J* = 8.6 Hz), 7.90 (d, 1H, *J* = 8.6 Hz), 7.88 (d, 1H, *J* = 8.6 Hz), 7.73 (d, 1H, *J* = 5.6 Hz), 7.02–7.08 (m, 5H), 5.60 (d, 1H, *J* = 12.9 Hz), 5.56 (d, 1H, *J* = 12.9 Hz), 5.00 (d, 1H, *J* = 8.3 Hz), 4.68 (q, 1H), 3.11 (2H) and 1.41 (s, 9H).

D-5F1 was prepared with the same procedure as that for **L-5F1** using Boc-D-Phe. **D-5F1** (162.9 mg, yield 94.6%); mp 60–61 °C.

4.3. Preparation of Boc-(phenylalanine)$_2$ methyl[5] thiaheterohelicene ester 5F2

5HM (99.0 mg, 0.3 mmol), Boc-Phe-Phe-OH (152 mg, 0.36 mmol), and DMAP (9.2 mg, 0.07 mmol) were dissolved in 1 ml of anhydrous dichloromethane. DCC (85.6 mg, 0.36 mmol) was added to the mixture at 0 °C. The mixture was continuously stirred until no residual **5HM** was detected by silica-gel TLC (ethyl acetate/hexane = 1/2). The same procedure for **5F1** synthesis gave orange solid. Recrystallization of this solid from ethanol gave pale yellow solid of 5F2 (116.7 mg, yield 53.3%). Mp 174–175 °C and *m*/*z* 743 (M^++Na^+) (calcd 720). Anal. Calcd for $C_{40}H_{36}N_2O_5S_3$: C, 66.64; H, 5.03; N, 3.89. Found: C, 66.68; H, 5.22; N, 3.87. ^{1}H NMR (270 Hz, $CDCl_3$): δ = 8.31 ppm (s, 1H), 8.24 (d, 1H, *J* = 5.4 Hz), 8.03 (d, 1H, *J* = 8.6 Hz), 7.96 (d, 1H, *J* = 8.6 Hz), 7.91 (d, 1H, *J* = 8.4 Hz), 7.89 (d, 1H, *J* = 8.6 Hz), 7.73 (d, 1H, *J* = 5.6 Hz), 6.86–7.22 (m, 10H), 6.30 (d, 1H, *J* = 7.1 Hz), 5.49 (ABq, 2H, *J* = 18.5 Hz), 4.85–4.88 (m, 2H), 3.01–3.08 (m, 4H) and 1.37 (s, 9H).

4.4. Preparation of Boc-(phenylalanine)$_3$ methyl[5] thiaheterohelicene ester 5F3

5F1 (60.8 mg, 0.1 mmol) was dissolved in 4 mol/l HCl 1,4-dioxane solution (0.6 ml) at 0 °C, then the solution was left at room temperature for 2 h. The solvent was evaporated under 30 °C. A small portion of anhydrous 1,4-dioxane was added and evaporated again. This procedure was repeated three times. After washing with anhydrous ethyl ether three times, **5F1–NH_2·HCl** was obtained. **5F1–NH_2·HCl** was dissolved in 0.3 ml anhydrous DMF, neutralized with 5% trimethylamine in 1,4-dioxane (0.2 ml, 0.1 mmol) at 0 °C, and then Boc-Phe-Phe-OH (49.4 mg, 0.12 mmol) was added. The same procedure as that for **5F2** was done using 0.1 mol/l DCC 1,4-dioxane solution at 0 °C to give orange solid. Recrystallization from acetonitrile produced pale yellow solid of **5F3** (29.9 mg, 34.4%). Mp 209–210 °C and *m*/*z* 890 (M^++Na^+) (calcd 868). Anal. Calcd for $C_{49}H_{45}N_3O_6S_3$: C, 67.80; H, 5.22; N, 4.84. Found: C, 67.30; H, 5.59; N, 4.42. ^{1}H NMR (270 Hz, $CDCl_3$): δ = 8.31 ppm (s, 1H), 8.25 (d, 1H, *J* = 5.6 Hz), 8.03 (d, 1H, *J* = 8.6 Hz), 7.95 (d, 1H, *J* = 8.6 Hz), 7.90 (d, 1H, *J* = 8.6 Hz), 7.88 (d, 1H, *J* = 8.4 Hz), 7.74 (d, 1H, *J* = 5.6 Hz), 6.89–7.30 (m, 15H), 6.37 (d, 1H, *J* = 7.8 Hz), 6.22 (br, 1H), 5.49 (ABq, 2H, *J* = 15.8 Hz), 4.75–4.82 (m, 2H), 4.55 (q, 1H, *J* = 6.8 Hz), 4.27 (q, 1H, *J* = 6.8 Hz), 2.87–3.11 (m, 6H) and 1.35 (s, 9H).

4.5. Preparation of Boc-(phenylalanine)$_3$-OMe F3

H-Phe-OMe·HCl (537.7 mg, 2.5 mmol) and triethylamine (0.35 ml, 2.5 mmol) were dissolved in 5 ml of anhydrous dichloromethane. Boc-Phe-Phe-OH (1034 mg, 2.5 mmol) and DCC (583.7 mg, 3 mmol) were added to the mixture at 0 °C, which was stirred for one night at room temperature. The same procedure as that for **5F1** was performed, giving white solid. The solid was recrystallized from acetone/water to give white solid of **F3** (861.2 mg, yield 60.2%). Mp 174–175 °C and *m*/*z* 574 (M+H) (calcd 573). Anal. Calcd for $C_{33}H_{39}N_3O_6$: C, 69.09; H, 6.85; N, 7.32. Found: C, 69.01; H, 6.91; N, 7.38. ^{1}H NMR (270 Hz, $CDCl_3$): δ = 6.99–7.16 ppm (m, 15H), 6.37 (d, 1H, *J* = 7.4 Hz), 4.79 (br, 1H), 4.69 (q, 1H, *J* = 7.4 Hz), 4.53 (q, 1H, *J* = 7.3 Hz), 4.29 (q, 1H, *J* = 6.8 Hz), 3.67 (s, 3H), 2.87–3.09 (m, 6H) and 1.37 (s, 9H).

4.6. Preparation of Boc-(phenylalanine)$_3$-OH

Boc-(phenylalanine)$_3$-OMe **F3** (581.6 mg, 1.0 mmol) was dissolved in 10 ml of warm methanol. 1 M sodium hydroxide (1.2 ml, 1.2 mmol) was added to the solution, which was cooled to room temperature with stirring for 5 h. After confirming no **5HM** remained by TLC, the solution was made neutral by 10% citric acid and then the solvent was removed. The residue was dissolved in ethyl acetate and washed with 3% HCl and water twice. Performing the same procedure as that for the synthesis of **5F1**, white solid was obtained. The solid was recrystallized from ethyl acetate/hexane to give white solid of Boc-(phenylalanine)$_3$-OH (369.2 mg, 60.1%). Mp 194–196 °C and *m*/*z* 560 (M+H) (calcd 559). Anal. Calcd for $C_{32}H_{37}N_3O_6$: C, 68.68; H, 6.66; N, 7.51. Found: C, 68.51; H, 6.80; N, 7.38. ^{1}H NMR (270 Hz, $CDCl_3$): δ = 7.03–7.29 ppm (m, 15H), 6.48 (d, 1H, *J* = 6.9 Hz), 4.92 (br, 1H), 4.58–4.68 (m, 2H), 4.38 (br, 1H), 3.20 (d, 1H, *J* = 5.6 Hz), 3.14 (d, 1H, *J* = 5.6 Hz, 2.86–3.03 (m, 6H) and 1.36 (s, 9H).

4.7. Preparation of Boc-(phenylalanine)$_4$-OMe F4

H-Phe-OMe·HCl (44.2 mg, 0.2 mmol), triethylamine (0.028 ml, 0.2 mmol), Boc-(phenylalanine)$_3$-OH (115.0 mg, 0.2 mmol), DCC (51.1 mg, 0.24 mmol), and 1.2 ml of anhydrous dichloromethane were used according to the same procedures of the synthesis of

Boc-(phenylalanine)$_3$-OMe to give white solid of **F4** (69.7 mg, 47.2%). Mp 183–184 °C and *m*/*z* 721 (M+H) (calcd 720). Anal. Calcd for $C_{42}H_{48}N_4O_7$: C, 69.98; H, 6.71; N, 7.77. Found: C, 69.96; H, 6.80; N, 7.15. ^{1}H NMR (270 Hz, $CDCl_3$): δ = 6.98–7.26 ppm (m, 20H), 6.56 (br, 1H), 6.34 (br, 1H), 4.74–4.76 (m, 2H), 4.50 (q, 1H, *J* = 6.4 Hz), 4.17 (q, 1H, *J* = 5.8 Hz), 4.03 (br, 1H), 3.69 (s, 3H), 2.84–3.12 (m, 8H) and 1.34 (s, 9H).

4.8. Preparation of Boc-(phenylalanine)$_4$ methyl[5] thiaheterohelicene ester 5F4

According to the same procedures for **5F3** using **5F1** (143.7 mg, 0.25 mmol), 1 ml of 4 M HCl in 1,4-dioxane, 5% triethylamine in 1,4-dioxane (0.5 ml, 0.25 mmol), Boc-(phenylalanine)$_4$-OH (168.8 mg, 0.03 mmol), 0.1 M DCC in 1,4-dioxane (3.0 ml, 0.3 mmol), and 0.6 ml of anhydrous DMF, orange solid was obtained. The solid was recrystallized from acetonitrile/ethanol, giving pale yellow solid of **5F4** (40.3 mg, 15.9%). Mp 218–219 °C and *m*/*z* 1037 (M^++Na^+) (calcd 1015). Anal. Calcd for $C_{58}H_{54}N_4O_7S_3$: C, 68.62; H, 5.36; N, 5.52. Found: C, 67.02; H, 5.31; N, 5.18. ^{1}H NMR (270 Hz, $CDCl_3$): δ = 8.33 ppm (s, 1H), 8.29 (d, 1H, *J* = 5.4 Hz), 8.03 (d, 1H, *J* = 8.4 Hz), 7.96 (d, 1H, *J* = 8.6 Hz), 7.89 (d, 1H, *J* = 8.4 Hz), 7.88 (d, 1H, *J* = 8.6 Hz), 7.76 (d, 1H, *J* = 5.6 Hz), 6.92–7.26 (m, 20H), 6.66 (br, 1H), 6.48 (br, 1H), 6.21 (d, 1H, *J* = 6.9 Hz), 5.52 (ABq, 2H, *J* = 11.2 Hz), 4.84 (q, 1H, *J* = 6.3 Hz), 4.68 (m, 2H), 4.44 (q, 1H, *J* = 6.8 Hz), 4.12 (q, 1H, *J* = 6.3 Hz), 2.76–3.18 (m, 8H) and 1.31 (s, 9H).

4.9. Preparation of Boc-(phenylalanine)$_5$ methyl[5]thiaheterohelicene ester 5F5

According to the same procedures for **5F3** using **5F2** (74.1 mg, 0.1 mmol), 0.6 ml of 4 M HCl in 1,4-dioxane, 5% triethylamine in 1,4-dioxane (0.2 ml, 0.1 mmol), Boc-(phenylalanine)$_3$-OH (67.8 mg, 0.12 mmol), 0.1 M DCC in 1,4-dioxane (1.2 ml, 0.12 mmol), and 0.4 ml of anhydrous DMF, orange solid was obtained. The solid was purified by HPLC (silica-gel column, chloroform), yielding pale yellow solid of **5F5** (66.1 mg, 55.3%). Mp 222–223 °C and *m*/*z* 1184 (M^++Na^+) (calcd 1162). Anal. Calcd for $C_{67}H_{63}N_5O_8S_3$: C, 69.23; H, 5.46; N, 6.02. Found: C, 68.43; H, 5.52; N, 6.04. ^{1}H NMR (270 Hz, $CDCl_3$): δ = 8.33 ppm (s, 1H), 8.29 (d, 1H, *J* = 5.8 Hz), 8.02 (d, 1H, *J* = 8.6 Hz), 7.94 (d, 1H, *J* = 8.6 Hz), 7.88 (d, 1H, *J* = 8.4 Hz), 7.77 (d, 1H, *J* = 5.6 Hz), 6.87–7.29 (m, 26H), 6.59 (br, 1H), 6.29 (d, 1H, *J* = 5.6 Hz), 5.53 (ABq, 2H, *J* = 11.7 Hz), 4.90 (q, 1H, *J* = 7.4 Hz), 4.69 (m, 2H), 4.58 (br, 1H), 4.30 (q, 1H, *J* = 5.8 Hz), 4.07–4.14 (m, 2H), 2.70–3.22 (m, 10H) and 1.27 (s, 9H).

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