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Helical Oligopeptides of a Quaternized Amino Acid with Tunable Chiral-Induction Ability and an Anomalous pH Response

Joonil Cho,[a],[b] Yasuhiro Ishida,*[b] and Takuzo Aida*[a]

Abstract: A series of octamer (8mer) and hexadecamer (16mer) oligopeptides of 4-aminopiperidine-4-carboxylic acid (Api) with L-leucine as a chiral auxiliary at their N- or C-termini were synthesized. Using circular dichroism spectroscopy, the conformational profiles of the peptides were systematically studied, which revealed that the α-helix formation ability of the peptides is determined by the combination of parameters including peptide length, state of the piperidine groups in the Api units, and position of the chiral auxiliary. When the piperidines are at the free-base state, the peptides show low propensity to form helical structures. However, the protonation and acylation of the piperidines enhance the formation of helical structures, where the order in helix formation ability is: protonated > acylated > free-base. In terms of peptide length, the 16mers generally show higher helix-formation ability than the corresponding 8mers, and one of the 16mers shows helicity at a highest level for the oligopeptides with similar length. It was also found that the sensitivity of the helical structure toward the state of the piperidines changes drastically, depending on the chiral auxiliary position; the N-terminal chiral peptides are more sensitive than the C-terminal chiral ones.

Introduction

The handedness of most helical biopolymers and synthetic polymers is determined by the absolute configuration of their constituent monomer units.[1,2] In sharp contrast to such static helices, some synthetic polymers are known to adopt dynamic helical conformations. Pioneering examples[3] include polyisocyanides, polymethacrylates, and polyisocyanates, whereas more-recent examples[4] feature polyacetylene derivatives. These dynamic helical polymers have attracted particular attention as chiroptical sensors, because their helical sense can switch in response to chiral stimuli or to the environment of the polymer.[1b,5]

In the 1980s, Toniolo and co-workers reported that a sufficiently long synthetic oligopeptide of 2-aminoisobutyric acid (Aib) shows a dynamic helical inversion in solution.[6,7] Although Aib is achiral, its oligomers adopt a helical conformation because of steric repulsion between its side chains.[8] More recently, it has been reported that, by incorporating chiral amino acids, the helical structure of oligomeric Aib can be biased to either a right- or left-handed conformation.[9–11] Although structural diversification has been achieved by replacing Aib with other α,α-dialkylamino acids,[12–14] most of these monomers are not amenable to side-chain functionalization, limiting their range of potential applications.

With these aspects in mind, we focused our attention on 4-aminopiperidine-4-carboxylic acid (Api),[15] which bears a reactive secondary amino group (Figure 1, center). Although some oligopeptides containing Api have been reported,[16] the conformational properties of Api oligopeptides had not been explored until our previous work on Api octamers (8mers).[17] We showed that Api 8mers with desired terminal functionalities can be synthesized by a solution-phase iterative method.

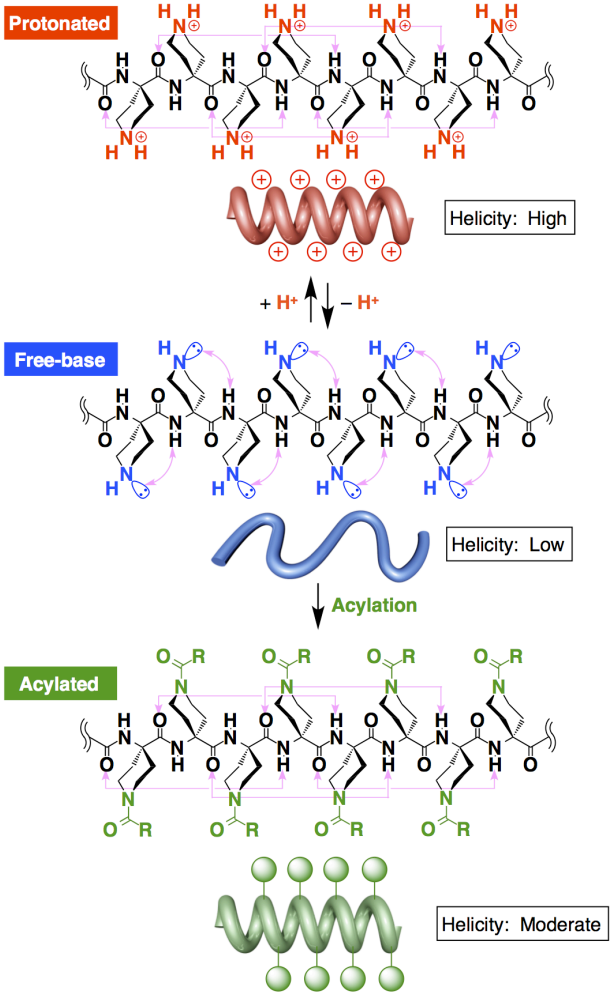

Figure 1. General tendency of conformational profiles of the Api peptides with three different states of the secondary amine groups in the piperidine groups: free-base (center), protonated (top), and acylated (bottom) states.

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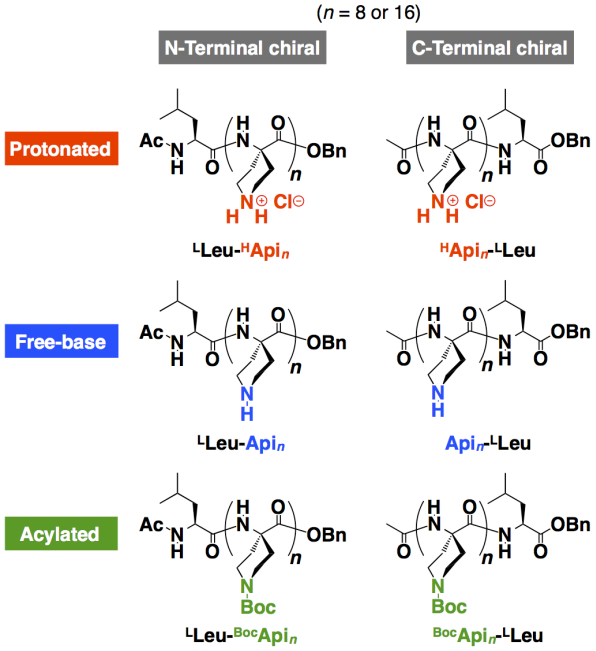

Figure 2. Molecular structures of Api 8mers and 16mers carrying an ᴸLeu moiety at the N- or C-terminus with various states of the secondary amine groups in the piperidine moieties: free-base (blue), protonated (red), and acylated (green) (Ac = acetyl; Boc = *tert*-butoxycarbonyl; Bn = benzyl).

Furthermore, we demonstrated that Api 8mers substituted with enantiopure leucine (L- or D-form; ᴸLeu or ᴰLeu) at their N-termini display an interesting change in conformation in response to changes in pH, in which protonation of the piperidine groups triggers a nonhelical-to-helical conformational transition (Figure 1, center to top). In sharp contrast to conventional basic peptides,[18] the Api 8mer is the first basic oligopeptide that adopts a helical conformation in acidic media only. As confirmed by ^{1}H NMR spectroscopy,[17] the conformational locking induced by the formation of a hydrogen-bonding network around the peptide backbone can be manipulated through involvement of the basic piperidine groups (Figure 1, center). In addition, it was preliminarily confirmed that the acylation of the piperidine groups also induce Api peptides to adopt an α-helical structure, because the acylation diminishes the basicity of the secondary amine lope pairs, thereby releasing the conformational locking (Figure 1, center to bottom).[19]

Despite these interesting observations, a number of questions about Api oligopeptides remain unanswered: (i) Which structural elements ensure the helical conformation?, (ii) How large is the helical content?, and (iii) How can the pH responsiveness be modulated? Another open question is (iv) Which of the protonation and acylation of the piperidine groups induce the helical structures more efficiently?

To address these issues, we have synthesized a series of Api octamers (8mers) and hexadecamers (16mers) with varying the state of the piperidine groups (Figure 2) and have compared their conformational profiles. To monitor the conformational properties of the Api peptides by circular dichroism (CD) spectroscopy, enantiopure Leu (ᴸLeu was mainly used) was incorporated at either of the N- or C-terminus of the Api peptides to bias the equilibrium of the two enantiomeric conformations toward one state. These systematic studies fully addressed the open questions mentioned above and they clearly demonstrated the potential utility of Api peptides as new motifs for the study of polymers and peptides with dynamic helical structures.

Results and Discussion

Synthesis of Api Peptides

The Fmoc-terminated peptide Fmoc(^{Boc}Api)$_8$OBn was synthesized by a solution-phase method, as previously reported (Scheme S1).[17] The target 8mers with N-terminal chiral auxiliary [Ac(^{L}Leu)(Api)$_8$OBn (= **^{L}Leu-Api$_8$**), Ac(^{L}Leu)(^{H}Api)$_8$OBn (= **^{L}Leu-^{H}Api$_8$**), and Ac(^{L}Leu)(^{Boc}Api)$_8$OBn (= **^{L}Leu-^{Boc}Api$_8$**)] and with C-terminal chiral auxiliary [Ac(Api)$_8$(^{L}Leu)OBn (= **Api$_8$-^{L}Leu**) Ac(^{H}Api)$_8$(^{L}Leu)OBn (= **^{H}Api$_8$-^{L}Leu**), and Ac(^{Boc}Api)$_8$(^{L}Leu)OBn (= **^{Boc}Api$_8$-^{L}Leu**)] were prepared from Fmoc(^{Boc}Api)$_8$OBn by the introduction of a chiral auxiliary at the N- and C-termini, conversion of the N-terminal end-capping group, and conversion of the piperidine groups in Api units, as shown in Scheme S2.

The target 16mers were synthesized by a similar route to the 8mers, but with some modifications because the corresponding precursor Fmoc(^{Boc}Api)$_{16}$OBn was not available. Condensation of Fmoc(^{Boc}Api)$_8$OH with H(^{Boc}Api)$_8$OBn was sluggish and was accompanied by the formation of unknown side-products due to the labile nature of the Fmoc group (Scheme S1) under the condensation conditions. In an alternative approach (Scheme S3), we condensed Ac(^{L}Leu)(^{Boc}Api)$_8$OH with H(^{Boc}Api)$_8$OBn to give Ac(^{L}Leu)(^{Boc}Api)$_{16}$OBn (= **^{L}Leu-^{Boc}Api$_{16}$**), which was then converted into Ac(^{L}Leu)(^{H}Api)$_{16}$OBn (= **^{L}Leu-^{H}Api$_{16}$**) and Ac(^{L}Leu)(Api)$_{16}$OBn (= **^{L}Leu-Api$_{16}$**). In a similar manner, the target 16mers with C-terminal chiral auxiliary [Ac(Api)$_{16}$(^{L}Leu)OBn (= **Api$_{16}$-^{L}Leu**), Ac(^{H}Api)$_{16}$(^{L}Leu)OBn (= **^{H}Api$_{16}$-^{L}Leu**), and Ac(^{Boc}Api)$_{16}$(^{L}Leu)OBn (= **^{Boc}Api$_{16}$-^{L}Leu**)] were prepared.

Circular Dichroism (CD) Spectroscopy of Api Peptides

Conformational features of the Api peptides were investigated by CD spectroscopy. For comparing the CD profiles of the Api peptides between the three states of the piperidine units (free-base, protonated, and acylated), MeOH was chosen as a solvent, because of its ability to dissolve all of these Api peptides (Figures 3, 4, and S1–S3). For comparing the helicity of the protonated Api peptides with other reported oligopeptides, aqueous HCl (pH 4–5) was used, because previous examples were usually measured in not MeOH but aqueous media (Table 1 and Figures S4 and S5). For the pH titration experiment of the protonated/free-base Api peptides, aqueous media were used, in order to control the pH values accurately (Figure 5). In all cases, CD intensity was converted into mean-residue molar ellipticity ($[\theta]_{MRE}$ = [θ]/number of amino acid residues).

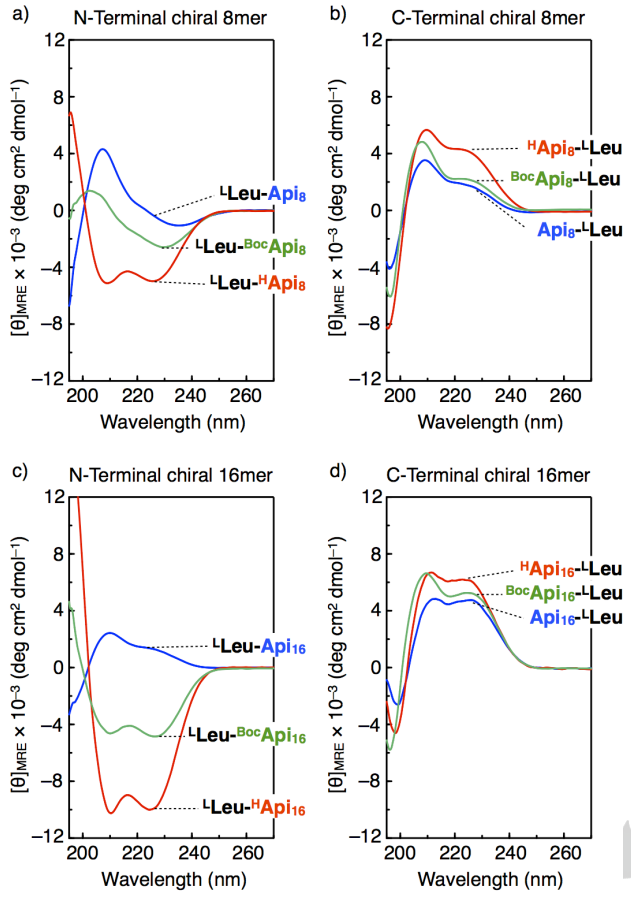

Figure 3. CD spectra in MeOH at 20 °C of the Api peptides with the free-base (**Api**$_n$, blue), protonated (^{H}**Api**$_n$, red), and acylated (^{Boc}**Api**$_n$, green) states (n = 8 or 16). a) N-terminal chiral 8mers. b) C-terminal chiral 8mers. c) N-terminal chiral 16mers. d) C-terminal chiral 16mers. For the concentrations of the Api peptides, see Experimental Section.

Artifacts such as the contamination of linear dichroism signals are likely to be excluded, because the D-forms of the Api peptides, synthesized from ^{D}Leu by the same routes as the L-forms, showed CD spectra that were essentially the mirror images of those of the L-forms (Figure S1). In addition, for the CD spectra of all the Api peptides, essentially no concentration dependence was observed, indicating that the aggregation of Api peptides is negligible in the present CD measurement conditions (Figures S2 and S3).

Chiroptical Profiles of Api Peptides (i): Effects of the State of the Piperidine Groups

We at first focused on the effects of the peptide length and the state of the piperidine groups, with fixing the chiral auxiliary position at the N-terminus (Figures 3a and 3c). CD spectra of the free-base peptides **^{L}Leu-Api$_8$** and **^{L}Leu-Api$_{16}$** show a single positive peak at around 210 nm, which are obviously different from the CD profiles of α-helices (Figures 3a and 3c, blue). As we clarified in our previous report by using H–D exchange as a probe,[17] the nonprotonated piperidine groups in **^{L}Leu-Api$_8$** and **^{L}Leu-Api$_{16}$** probably interact with neighboring amide NH groups and hinder their hydrogen bonding with carbonyl groups along the peptide backbone (Figure 1, center). In sharp contrast, the protonated oligopeptides **^{L}Leu-^{H}Api$_8$** and **^{L}Leu-^{H}Api$_{16}$** showed CD patterns typical of a right-handed α-helical structure (Figures3a and 3c, red); the two characteristic Cotton effects at 211 (π–π*) and 225 nm (n–π*) were explicit, and the ratio of their intensities was almost unity.[20] As shown in our previous study, the formation of an α-helical structure was unambiguously confirmed for the analogous Api 8mer [Ac(^{H}Api)$_8$NHMe] by a ^{1}H NMR saturation-transfer experiment and by ^{1}H–^{1}H correlations evaluated by 2D rotational Overhauser effect spectroscopy (ROESY).[17] In the protonated state, the hydrogen-bond accepting sites in the piperidine groups are occupied by protons, so that the amide NH groups in the peptide backbone can form the hydrogen-bonding networks characteristic of α-helical structures (Figure 1, top). As described in the introduction, the response of Api peptides toward acidic and basic conditions is opposite to that of conventional basic peptides.[18]

Table 1. Helicities of typical canonical oligopeptides with high α-helical tendencies compared with those of the Api oligopeptides

Peptide	Number of residues	$[\theta]_{MRE\_n\text{-}\pi^*}$[a] (deg cm^2 dmol^{-1})	Helicity[b] (%)
$DPAEA_2KA_3GR$[c]	12	–7900	25
$S_2DVSTAQA_3YKLHED$[d]	17	–9900	29
^{L}Leu-^{H}Api$_8$	9	–5000[e]	17
^{L}Leu-^{H}Api$_{16}$	17	–11000[e]	32

[a] Mean residue molar ellipticity values at the absorption band of amide n–π* transition. [b] Estimated according to the equation reported in ref. 21. Percentage helicity = 100 × $[\theta]_{MRE\_n\text{-}\pi^*}$ / $^{max}[\theta]_{MRE\_n\text{-}\pi^*}$, where $^{max}[\theta]_{MRE\_n\text{-}\pi^*} = -40000 \times [1 - (2.5/m)]$ and m = number of amino acid residues. [c] Ref. 21. [d] Ref. 22. [e] Calculated from the CD spectrum measured at [peptide] = 30 μM in aqueous HCl (pH 4.0) at 0 °C.

Although the CD spectra of the protonated peptides **^{L}Leu-^{H}Api$_8$** and **^{L}Leu-^{H}Api$_{16}$** were quite similar in pattern, they were notably different in intensity. The $[\theta]_{MRE}$ of **^{L}Leu-^{H}Api$_{16}$** was about twice that of **^{L}Leu-^{H}Api$_8$** (Figures 3a and 3c, red). When the medium was changed from MeOH to aqueous HCl (pH 4.0), the difference in $[\theta]_{MRE}$ was further enhanced; the $[\theta]_{MRE}$ of **^{L}Leu-^{H}Api$_{16}$** was 2.3 times greater than that of **^{L}Leu-^{H}Api$_8$** (Figure S4) These observations show that chain elongation significantly stabilizes the α-helical conformation of Api oligopeptides. Note that the average fractional helicity of **^{L}Leu-^{H}Api$_{16}$**, as estimated from the CD intensity at 225 nm (n–π*), is comparable to or even greater than that of a typical α-helical oligopeptide (17 residues) specifically designed to form a stable α-helix (Table 1).[21–23] Considering that **^{L}Leu-^{H}Api$_{16}$** has only one stereogenic center at its N-terminus, whereas the reference oligopeptides consist exclusively of enantiopure amino acids, we can conclude that the present Api oligopeptide realizes a highly efficient transfer of chiral information.

As described in the introduction, acylation of the piperidine groups is also anticipated to induce helical structures (Figure 1, center to bottom). Indeed, the acylated 16mer **^{L}Leu-^{Boc}Api$_{16}$**, bearing *tert*-butoxycarbonyl units on the secondary amines of the piperidine groups, displayed CD bands at 210 and 225 nm, characteristic of an α-helical conformation (Figure 3c, green). Contrary to this, the acylated 8mer **^{L}Leu-^{Boc}Api$_{8}$** displayed CD profiles quite different from α-helices (Figure 3a, green).[19] Therefore, the effect of acylation on helix induction (Figure 1, center to bottom) is more dependent on the length of the oligopeptide chain than on protonation (Figure 1, center to top). In addition, the helicity induced by acylation is lower than that induced by protonation (Table 1).

In the following sections, we focus on the 16mers, because the helix-formation ability of the 16mers superior to the 8mers would make the discussion simpler.

Chiroptical Profiles of Api Peptides (ii): Effects of the Position of the Chiral Auxiliary

The conformational features of the C-terminal chiral 16mers were also studied in the similar manner to the N-terminal chiral ones (Figure 3d). At the protonated state, the C-terminal chiral 16mer (**^{H}Api$_{16}$-^{L}Leu**) adopted a left-handed α-helical structure characterized with two positive Cotton effects (Figure 3d, red), whose handedness was opposite to the N-terminal chiral 16mer (**^{L}Leu-^{H}Api$_{16}$**).[24] Considering that these 16mers possess chiral auxiliaries based on the same amino acid (^{L}Leu), the inconsistency of their helical handedness apparently seems strange, but similar helicity inversion was reported for a pair of Aib oligopeptides, having an L-amino acid at either of the N- or C-terminus.[25] It should also be noted that the helical handedness is determined not only by the position of chiral auxiliary but by the balance of several parameters; even a slight modification of the C-terminal chiral auxiliary, replacing the endcapping group from the ester to the corresponding amide, caused the helicity inversion, giving the same handedness to the N-terminal chiral peptide (Figure S5).[25]

With varying the state of the piperidine groups, the helicity of the C-terminal chiral 16mers changed only slightly (Figure 3d), unlike the change of the N-terminal chiral 16mers (Figure 3c). Indeed, the $[\theta]_{MRE}$ of C-terminal chiral 16mer in the acylated state (**^{Boc}Api$_{16}$-^{L}Leu**; Figure 3d, green) is slightly lower than that in the protonated state (**^{H}Api$_{16}$-^{L}Leu**; Figure 3d, red). Noteworthy, the C-terminal chiral 16mer retained certain helicity even at the free-base state (**Api$_{16}$-^{L}Leu**; Figure 3d, blue), although the helicity is the lowest among the three states. Overall, these observations imply that the sensitivity of the present helical structures toward the state of the piperidine unit can be modulated by the position of the chiral auxiliary; the C-terminal chiral 16mer is less sensitive than the N-terminal chiral 16mer. As a further confirmation, the 8mers also showed the similar tendency (Figures 3a and 3b).

Although the studies on detailed mechanism are now ongoing in our group, these observations can be elucidated by the following hypothesis. In the free-base form of the N-terminal chiral and the C-terminal chiral 16mers, one or two piperidine groups located near to the chiral auxiliary can interact with the adjacent amide NH groups in the peptide backbone (Figures S6, blue), so that the chiral information transfer from the chiral auxiliary to the achiral Api oligomers (Figures S6, red) is 'disrupted'. In the N-terminal chiral 16mer, the piperidine groups in charge of the 'disrupting' are on the 3rd and 4th residue from the N-terminus (Figure S6a, blue). Meanwhile, the 'disrupting' residue from the C-terminus (Figure S6b, blue), whose secondary amine, located very near to the terminus, is exposed to exterior solvent and therefore prone to be solvated. Accordingly, the 'disrupting' of the C-terminal chiral 16mer is partially decelerated even at the free-base state.

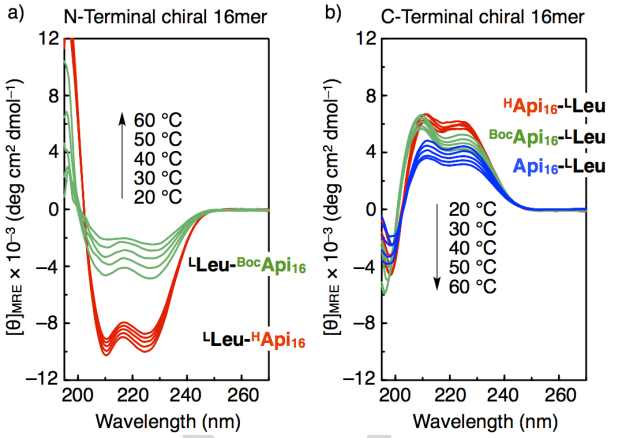

Figure 4. CD spectra in MeOH of Api 16mers at various temperatures from 20 to 60 °C: a) **^{L}Leu-^{H}Api$_{16}$** (red) and **^{L}Leu-^{Boc}Api$_{16}$** (green) and b) **^{H}Api$_{16}$-^{L}Leu** (red), **^{Boc}Api$_{16}$-^{L}Leu** (green), and **Api$_{16}$-^{L}Leu** (blue). For the concentrations of the Api peptides, see Experimental Section.

Chiroptical Profiles of Api Peptides (iii): Response to Changes in Temperature

With five α-helical 16mers (**^{L}Leu-^{H}Api$_{16}$**, **^{L}Leu-^{Boc}Api$_{16}$**, **^{H}Api$_{16}$-^{L}Leu**, **^{Boc}Api$_{16}$-^{L}Leu**, and **Api$_{16}$-^{L}Leu**) in hand (Figures 3c and 3d), we investigated the stability of their conformations to changes in temperature (Figure 4).

To clarify the effects of piperidine state on the heat tolerance of the helical structures, we at first focus on the data of the C-terminal chiral 16mers, which can adopt helical structures at any state of the piperidine groups (Figure 4b). Upon heating from 20 to 60 °C, the protonated 16mer (**^{H}Api$_{16}$-^{L}Leu**) showed relatively small change in its CD profiles (Figure 4b, red), where $[\theta]_{MRE}$ slightly decreased (9%) with respect to that of the original state at 20 °C. Meanwhile, the acylated (**^{Boc}Api$_{16}$-^{L}Leu**) and free-base 16mers (**Api$_{16}$-^{L}Leu**) were more prone to heat-induced denaturation (Figure 4b, green); on heating from 20 to 60 °C, the $[\theta]_{MRE}$ of the acylated and free-base 16mers decreased by 19% and 28%, respectively. Thus, the heat tolerance of the helical structures of the Api peptides is in the following order: protonated > acylated > free-base. As a confirmation for the generality of the above rule, the N-terminal chiral 16mers (**^{L}Leu-**

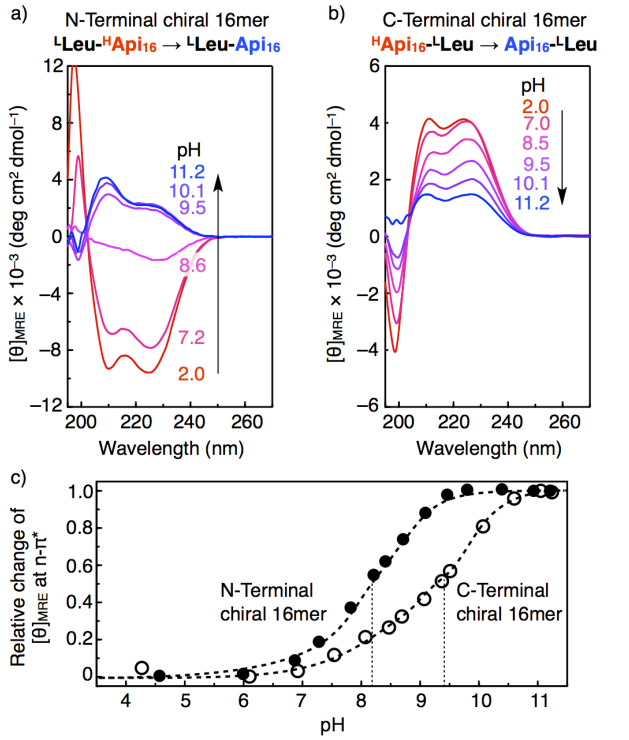

Figure 5. CD spectra ([peptide] = 30 μM) in aqueous media at 20 °C of a) **ᴸLeu-ᴴApi$_{16}$** and b) **ᴴApi$_{16}$-ᴸLeu** upon titration from pH 2 to 11. (c) Plots of the relative changes in $[\theta]_{MRE}$ (= $\{[\theta]_{MRE} - [\theta]_{MRE\_pH2}\}$ / $\{[\theta]_{MRE\_pH11} - [\theta]_{MRE\_pH2}\}$) at 226 nm as a function of the pH value. The broken lines indicate the pH midpoints.

ᴴApi$_{16}$ and **ᴸLeu-^{Boc}Api$_{16}$**) also showed the same tendency in heat tolerance (protonated > acylated; Figure 4a), although the data of the free-base state (**ᴸLeu-Api$_{16}$**) are missing because of its poor helix-formation ability. Relatively low tolerance of the helical structure acylated peptides (**ᴸLeu-^{Boc}Api$_{16}$** and **^{Boc}Api$_{16}$-ᴸLeu**) might be attributed to the steric hindrance of the Boc group.

Chiroptical Profiles of Api Peptides (iv): Response to Changes in pH

We next investigated the pH responsiveness of the Api 16mers (**ᴴApi$_{16}$** ⇌ **Api$_{16}$**) by a titration method. Thus, the protonated 16mers (**ᴸLeu-ᴴApi$_{16}$** or **ᴴApi$_{16}$-ᴸLeu**) were dissolved in aqueous HCl (pH 2.0), and the resultant solutions were titrated with aqueous NaOH while the resulting conformational change was monitored by CD spectroscopy. At the initial and final states of the titration, the CD profiles of the N-terminal chiral and the C-terminal chiral 16mers were in consistent with the analogous measurement in MeOH (Figures 3c and 3d).[26] On increasing pH from 2.0 to 11.2, the N-terminal chiral 16mer underwent a drastic helical-to-nonhelical structural transition (Figure 5a), while the C-terminal chiral 16mer just decreased its helicity (Figure 5b).

The pH-titration plot, *i.e.* the plot of the relative changes in the CD intensity at 226 nm as a function of the pH (Figure 5c), gives a more profound insight on the difference in pH responsiveness between the N-terminal chiral and the C-terminal chiral 16mers. On increasing the pH from 2.0 to 11.2, the N-terminal chiral 16mer underwent the structural transition at an earlier stage (closed circles) than the C-terminal chiral 16mer (open circles). Indeed, the pH midpoint for the N-terminal chiral 16mer is 8.1, while that of the C-terminal chiral 16mer is 9.4. Such a notable difference in pH responsiveness between the N-terminal chiral and the C-terminal chiral 16mers might be elucidated by difference in the environment of the 'disrupting' piperidines, as described above (Figure S6).[27]

Conclusions

A series of Api 8mers and 16mers were prepared as a new motif for the study of polymers and peptides with dynamic helical structures, and their conformational profiles were investigated with varying the peptide length, the state of the piperidine groups in the Api units, and the position of chiral auxiliary. These systematic studies revealed the following facts.

(i) Effect of peptide length: Chain elongation of the Api peptides enhances the tendency to form an α-helix (Figure 3). The 16mers generally show higher helix formation ability than the corresponding 8mers, and one of the 16mers shows a helicity of 32%, which is at a highest level for the oligopeptides with similar length (Table 1).

(ii) Effect of the state of the piperidine groups: Depending on the state of the piperidine groups, helix formation ability of the Api peptides changes as follows: protonated > acylated > free-base (Figure 3). Heat tolerance of the helical structures also changes in the same manner (Figure 4).

(iii) Effect of the position of the chiral auxiliary [1]: The position of chiral auxiliary affects the helical handedness (Figure 3). In the protonated state of the piperidines, the N-terminal chiral and the C-terminal chiral peptides adopt right- and left-handed helical structures, respectively. It should also be noted that the helical handedness is determined not only by the position of chiral auxiliary but by the balance of several parameters, including the structure of the endcapping groups (Figure S5).[25]

(iv) Effect of the position of the chiral auxiliary [2]: The position of the chiral auxiliary affects the sensitivity of the helical structures toward the state of the piperidine groups. The N-terminal chiral peptides are more sensitive than the C-terminal chiral ones (Figure 3).

(v) Effect of the position of the chiral auxiliary [3]: The position of the chiral auxiliary affects pH responsiveness of the helical structures (Figure 5). For the structural transition induced by the increase of pH, the C-terminal chiral peptide shows higher pH midpoint (pH 9.4) than the N-terminal chiral one (pH 8.1).

As described in the introduction, the Api peptides are the first basic oligopeptide that adopt a helical conformation in acidic

media exclusively. The conformational features of the 16mers clarified here, which are notably improved from those of the 8mers, should expand the utility of Api peptides. Furthermore, pH responsiveness of the Api peptides can be modulated by the position of the chiral auxiliary unit (N- or C-terminus). Considering these features, as well as the potential of the secondary amines in the piperidine groups for modification, such as acylation, alkylation, quaternization, metal coordination, *etc.*, the Api peptides developed here should serve as versatile precursors for various functionalized helical peptides.

Experimental Section

Materials and Methods

All reagents were purchased from Kanto Kagaku, Aldrich, Tokyo Kasei, and Wako Pure Chemical. Unless otherwise noted, they were used as received. For column chromatography, Wakogel silica C-300HG (particle size 40–60 μm) or C-400HG (particle size 20–40 μm) was used. Analytical TLC was performed with 0.25-mm thick silica gel 60F plates with a fluorescent indicator (254 nm).

^{1}H and ^{13}C NMR spectra were recorded on a JEOL model GSX270 spectrometer, operating at 270.05 and 67.80 MHz for ^{1}H and ^{13}C NMR, respectively, and a JEOL model JNM-ECA500 spectrometer operating at 500.16 MHz and 125.77 MHz for ^{1}H and ^{13}C NMR, respectively. Chemical shifts were determined with respect to partially or non-deuterated solvents [$(CH_3)_4Si$] as internal references. Electronic absorption (UV-vis) and circular dichroism (CD) spectra were recorded on a JASCO model U-best V-560 spectrometer and a JASCO model J-820 spectropolarimeter, respectively. High-resolution matrix-assisted laser desorption/ionization TOF-MS (HR-MALDI-TOF-MS) spectra were recorded on a Bruker model AutoFlex Speed mass spectrometer on a positive mode by using poly(ethylene glycol) (M_n = 1000, 2000, and 4000) as an internal reference, sodium trifluoroacetate as a protonation reagent, and a mixture of α-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) as a matrix. Ac($^{L/D}$Leu)(^{Boc}Api)$_8$OBn (= **$^{L/D}$Leu-^{Boc}Api$_8$**), Ac($^{L/D}$Leu)(Api)$_8$OBn (= **$^{L/D}$Leu-Api$_8$**), Ac($^{L/D}$Leu)(^{H}Api)$_8$OBn (= **$^{L/D}$Leu-^{H}Api$_8$**), and H(^{Boc}Api)$_8$OBn were synthesized according to the reported methods[17] with minor modification in the deprotection step of benzyl ester of Fmoc(^{Boc}Api)$_n$OBn (n = 2, 4, 8).[28]

Peptide Synthesis

The synthesis and characterization of the L-form peptides are described here. The D-form peptides were synthesized in the same methods to their antipodes, by using the starting materials with the corresponding stereochemistry.

Ac(^{L}Leu)(^{Boc}Api)$_8$OH

To a solution of Ac(^{L}Leu)(^{Boc}Api)$_8$OBn (414 mg, 0.20 mmol) in MeOH (6 mL) was added 10 wt% Pd/C (414 mg) at rt, and the resultant mixture was degassed and purged with hydrogen. After being stirred at rt for 12 h, the catalyst was filtered off through a Celite pad, and the solid was washed with a mixture of CH_2Cl_2/MeOH (10:1, v/v). The filtrate and washing were combined and concentrated under reduced pressure to dryness. The resultant solid was suspended in hexane, and then filtered and washed with hexane. The solid residue was dried under reduced pressure to afford Ac(^{L}Leu)(^{Boc}Api)$_8$OH as a white solid (368 mg, 186 μmol, 93%). ^{1}H NMR (500.16 MHz; CD_3CN; 50 °C): δ 7.66 (brs, 1H), 7.55 (brs, 1H), 7.52 (s, 1H), 7.46 (s, 1H), 7.44 (s, 1H), 7.41 (brs, 1H), 7.27 (s, 1H), 7.12 (s, 1H), 3.92 (brs, 1H), 3.85–2.55 (m, 32H), 2.40–1.85 (m, 32H), 1.70–1.52 (m, 3H), 1.43–1.38 (m, 72H), 0.95 (d, J = 6.3 Hz, 3H), 0.93 (d, J = 6.3 Hz, 3H) ppm; ^{13}C NMR (125.77 MHz; CD_3CN; 50 °C): δ 176.33, 176.30, 176.08, 175.76, 175.54, 175.21, 155.66, 155.63, 155.55, 155.44, 155.39, 80.35, 80.29, 80.23, 80.18, 80.11, 80.05, 50.43, 59.24, 59.22, 59.00, 58.84, 58.60, 58.57, 58.49, 41.2–40.2, 28.90, 28.87, 28.84, 25.73, 23.30, 22.94 ppm; HR-MALDI-TOF-MS calcd. for $C_{96}H_{159}N_{17}O_{27}Na$ ([M + Na]$^+$) 2005.1484, found 2005.1486.

Ac(^{L}Leu)(^{Boc}Api)$_{16}$OBn (= **^{L}Leu-^{Boc}Api$_{16}$**)

To a stirred CH_2Cl_2 solution (1 mL) of Ac(^{L}Leu)(^{Boc}Api)$_8$OH (198 mg, 0.10 mmol) at 0 °C, were added HOAt (14 mg , 0.10 mmol), HATU (38 mg, 0.10 mmol), and TMP (40 μL, 0.30 mmol) at 0 °C, and the mixture was stirred for 3 h at 0 °C. Then, H(^{Boc}Api)$_8$OBn (192 mg, 0.10 mmol) was added at 0 °C to the reaction mixture. After being stirred at 0 °C for 30 min, the reaction mixture was allowed to warm to rt and stirred for 4 days. Then, the solution was diluted with CH_2Cl_2 (10 mL), washed with aqueous HCl (1 M, 3 × 10 mL) and brine (10 mL), dried over $MgSO_4$, and evaporated to dryness under a reduced pressure at rt. The residue was subjected to silica gel column chromatography eluted with AcOEt/$CHCl_3$ (1:1–2:1, v/v) to afford Ac(^{L}Leu)(^{Boc}Api)$_{16}$OBn as a white solid (136 mg, 35 μmol, 35%). ^{1}H NMR (500.16 MHz; CD_3CN; 50 °C): δ 8.05 (brs, 1H), 7.80 (s, 2H), 7.76 (s, 1H), 7.73 (s, 1H), 7.69 (s, 1H), 7.64 (s, 1H), 7.62 (s, 1H), 7.57 (s, 1H), 7.46 (s, 1H), 7.42 (s, 1H), 7.38–7.29 (m, 5H), 7.28 (s, 1H), 7.12 (s, 2H), 6.76 (brs, 1H), 5.05 (s, 2H), 4.21 (m, 1H), 3.97–2.71 (m 64H), 2.24–1.70 (m, 64H), 1.97 (s, 3H), 1.70–1.52 (m, 3H), 1.45–1.37 (m, 144H), 0.96 (d, 6H) ppm; ^{13}C NMR (125.77 MHz; CD_3CN; 50 °C): δ 176.39, 176.31, 176.19, 176.01, 175.95, 175.89, 174.87, 174.71, 174.47, 174.41, 155.75, 155.68, 155.49, 137.58, 129.51, 129.19, 80.41, 80.36, 80.32, 80.24, 80.21, 80.18, 80.15, 80.10, 80.06, 80.02, 79.97, 79.91, 67.55, 59.64, 59.55, 59.03, 58.97, 58.70, 58.68, 58.57, 58.54, 58.45, 58.42, 57.42, 41.75–39.07, 35.93–34.65, 30.59–29.88, 25.79, 23.51, 23.39, 22.04 ppm; HR-MALDI-TOF-MS calcd. for $C_{191}H_{309}N_{33}O_{51}Na$ ([M + Na]$^+$) 3904.2492, found 3904.2426.

Ac(^{L}Leu)(^{H}Api)$_{16}$OBn (= **^{L}Leu-^{H}Api$_{16}$**)

To a stirred AcOEt solution (5 mL) of Ac(^{L}Leu)(^{Boc}Api)$_{16}$OBn (154 mg, 40 μmol) was introduced HCl gas over 15 min at rt, and the reaction mixture was stirred at rt for 30 min. The precipitate in the resultant mixture was collected by filtration, washed with hexane, and dried overnight at 60 °C under a reduced pressure to afford Ac(^{L}Leu)(^{H}Api)$_{16}$OBn as a white hygroscopic solid (114 mg, 40 μmol, quant). ^{1}H NMR (500.16 MHz; CD_3OD; 25 °C): δ 8.91(s, 1H), 8.88 (s, 1H), 8.53 (s, 1H), 8.41 (s, 1H), 8.39 (s, 1H), 8.22 (s, 1H), 8.18 (s, 1H), 8.12 (brs, 1H), 8.06 (s, 3H), 8.00 (s, 1H), 7.94 (s, 1H), 7.90–7.80 (m, 3H), 7.61 (s, 1H), 7.45–7.35 (m, 5H), 5.19 (ABq, 2H, $\Delta\nu_{AB}$ = 15.3 Hz, J_{AB} = 12.0 Hz), 3.95 (brs, 1H), 3.75–2.92 (m, 64H), 2.22 (s, 3H), 2.87–2.12 (m, 64H), 1.85–1.62 (m, 3H), 1.07 (d, J = 6.3 Hz, 3H), 1.01 (d, J = 6.3 Hz, 3H) ppm; HR-MALDI-TOF-MS calcd. for $C_{111}H_{181}N_{33}O_{19}Na$ ([M – 16HCl + Na]$^+$) 2303.4104, found 2303.4090.

Ac(^{L}Leu)(Api)$_{16}$OBn (= **^{L}Leu-Api$_{16}$**)

To a stirred aqueous solution (0.1 mL) of Ac(^{L}Leu)(^{H}Api)$_{16}$OBn (57 mg, 20 μmol) was added an aqueous solution of methanolic KOH solution (0.1 M, 3.3 mL) at rt. After being stirred at rt for 5 min, the reaction mixture was concentrated to dryness under a reduced pressure at rt. The resultant residue was suspended in MeOH (1 mL) and filtrated through a hydrophobic porous membrane (pore diameter = 0.20 μm, DISMIC–31jp). The filtrate was concentrated to dryness under a reduced pressure at rt to afford Ac(^{L}Leu)(Api)$_{16}$OBn as a white solid (46 mg, 20 μmol, quant). ^{1}H NMR (500.16 MHz; CD_3OD; 25 °C): δ 7.39–7.21 (m, 5H), 5.11 (ABq, 2H, $\Delta\nu_{AB}$ = 13.3 Hz, J_{AB} = 12.6 Hz), 3.98 (brs, 1H), 3.25 (m, 64H), 2.03 (s, 3H), 2.45–1.77 (m, 64H), 1.85–1.62 (m, 3H), 1.06 (d, J = 6.9 Hz, 3H), 0.99 (d, J = 6.3 Hz, 3H) ppm; HR-MALDI-TOF-MS calcd. for $C_{111}H_{181}N_{33}O_{19}Na$ ([M + Na]$^+$) 2303.4104, found 2303.4144.

Fmoc(^{Boc}Api)$_8$(^{L}Leu)OBn

By following the same procedure used for the preparation of Ac(^{Boc}Api)$_8$(^{L}Leu)OBn, Fmoc(^{Boc}Api)$_8$OH (377 mg, 184 μmol) and TsOH•H(^{L}Leu)OBn (73 mg, 184 μmol) were converted to

Fmoc(^{Boc}Api)$_8$(^{L}Leu)OBn as a white solid (352 mg, 156 µmol, 85%). ^{1}H NMR (500.16 MHz; $CDCl_3$; 50 °C): δ 7.77 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 7.5 Hz, 2H), 7.43 (m, 2H), 7.36–7.26 (m, 7H), 7.22 (s, 1H), 7.16 (s, 1H), 7.13 (s, 1H), 7.07 (brs, 2H), 6.77 (brs, 1H), 5.84 (brs, 1H), 5.09 (m, 2H), 4.65 (brs, 1H), 4.57 (m, 2H), 4.21 (t, J = 6.0 Hz, 1H), 4.03–2.50 (m, 32H), 2.37–1.56 (m, 35H), 1.52–1.38 (m, 72H), 0.90 (m, 6H) ppm; ^{13}C NMR (125.77 MHz; $CDCl_3$; 50 °C): δ 174.83, 174.76, 174.62, 174.58, 174.35, 173.31, 172.94, 165.74, 156.67, 154.69, 154.58, 154.53, 154.47, 154.33, 154.28, 154.26, 143.01, 142.97, 141.52, 141.46, 135.89, 128.38, 128.14, 128.12, 128.04, 127.90, 127.21, 124.56, 120.35, 80.41, 80.27, 80.15, 80.05, 79.93, 79.67, 79.45, 79.28, 67.13, 66.58, 58.86, 58.72, 58.06, 57.95, 57.63, 57.59, 57.54, 52.06, 47.24, 40.17–38.30, 34.74–33.37, 28.55–28.31, 24.51, 22.77, 21.67 ppm; HR-MALDI-TOF-MS calcd. for $C_{116}H_{173}N_{17}O_{28}Na$ ([M + Na]$^+$) 2275.2528, found 2275.2499.

H(^{Boc}Api)$_8$(^{L}Leu)OBn

To a stirred THF solution (3 mL) of Fmoc(^{Boc}Api)$_8$(^{L}Leu)OBn (338 mg, 150 µmol) was added diethylamine (0.3 mL, 3 mmol) at rt, and the reaction mixture was stirred at rt for 3 h. The reaction mixture was then evaporated to dryness under a reduced pressure at rt. The residue was chromatographed with CH_2Cl_2/MeOH (100:0–100:8, v/v) as an eluent, to allow the isolation of H(^{Boc}Api)$_8$(^{L}Leu)OBn (293 mg, 144 µmol, 96%). ^{1}H NMR (500.16 MHz; $CDCl_3$; 50 °C): δ 8.91 (brs, 1H), 7.44 (s, 1H), 7.42 (s, 1H), 7.40 (s, 1H), 7.33–7.26 (m, 5H), 7.21 (s, 1H), 7.05 (s, 1H), 6.55 (s, 1H), 5.11 (ABq, 2H, $\Delta\nu_{AB}$ = 12.6 Hz, J_{AB} = 12.6 Hz), 4.59 (m, 1H), 4.04–2.65 (m, 32H), 2.39–1.53 (m, 35H), 1.50–1.41 (m, 72H), 0.91 (d, J = 4.1 Hz, 3H), 0.90 (d, J = 4.1 Hz, 3H) ppm; ^{13}C NMR (125.77 MHz; $CDCl_3$; 50 °C): δ 178.66, 174.64, 174.45, 174.38, 173.90, 173.81, 173.63, 173.20, 154.69, 154.62, 154.59, 154.42, 136.23, 128.36, 128.04, 127.93, 80.77, 80.69, 80.52, 80.20, 79.79, 79.53, 79.23, 66.42, 59.04, 58.87, 58.09, 58.02, 57.80, 57.44, 57.25, 56.39, 51.39, 40.49–38.26, 34.68–33.75, 29.43, 28.57–28.35, 24.52, 23.02, 21.53 ppm; HR-MALDI-TOF-MS calcd. for $C_{101}H_{163}N_{17}O_{26}Na$ ([M + Na]$^+$) 2053.1847, found 2053.1816.

Ac(^{Boc}Api)$_8$(^{L}Leu)OBn (= **^{Boc}Api$_8$-^{L}Leu**)

To a stirred CH_2Cl_2 solution (1 mL) of H(^{Boc}Api)$_8$(^{L}Leu)OBn (145 mg, 71 µmol) and DIPEA (14 µL, 78 µmol) was added acetic anhydride (8 µL, 78 µmol) at 0 °C, and the mixture was allowed to warm to rt and stirred for 12h. Then, the solution was diluted with CH_2Cl_2 (10 mL), washed successively with hydrochloric acid (1 M, 3 x 10 mL) and brine (10 mL), dried over MgSO4, and concentrated to dryness under a reduced pressure at rt. The residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (100/5–100/8 in v/v) as an eluent, to afford Ac(^{Boc}Api)$_8$(^{L}Leu)OBn as a white solid (146 mg, 70 µmol, 99%). ^{1}H NMR (500.16 MHz; CD_3OD; 50 °C): δ 7.39–7.26 (m, 5H), 5.11 (ABq, 2H, $\Delta\nu_{AB}$ = 12.0 Hz, J_{AB} = 12.1 Hz), 4.50 (m, 1H), 4.04–2.63 (m, 32H), 2.52–1.69 (m, 35H), 1.61–1.56 (m, 3H), 1.51–1.39 (m, 72H), 0.93 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.3 Hz, 3H) ppm; HR-MALDI-TOF-MS calcd. for $C_{103}H_{165}N_{17}O_{27}Na$ ([M + Na]$^+$) 2095.1953, found 2095.1930.

Ac(^{H}Api)$_8$(^{L}Leu)OBn (= **^{H}Api$_8$-^{L}Leu**)

By following the same procedure used for the preparation of Ac(^{L}Leu)(^{H}Api)$_{16}$OBn, Ac(^{Boc}Api)$_8$(^{L}Leu)OBn (112 mg, 54 µmol) was converted into Ac(^{H}Api)$_8$(^{L}Leu)OBn as a white hygroscopic solid (84 mg, 54 µmol, quant.). ^{1}H NMR (500.16 MHz; CD_3OD; 25 °C): δ 8.94 (brs, 1H), 8.76 (s, 1H), 8.28 (s, 1H), 8.03 (brs, 1H), 7.90 (s, 1H), 7.79 (s, 1H), 7.56 (brs, 1H), 7.47–7.28 (m, 5H), 5.15 (ABq, 2H, $\Delta\nu_{AB}$ = 12.3 Hz, J_{AB} = 12.1 Hz), 4.57 (m, 1H), 3.82–2.91 (m, 32H), 2.86–1.94 (m, 35H), 1.84 (m, 2H), 1.63 (m, 1H), 0.94 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 6.9 Hz, 3H) ppm; HR-MALDI-TOF-MS calcd. for $C_{63}H_{101}N_{17}O_{11}Na$ ([M – 8HCl + Na]$^+$) 1294.7759, found 1294.7726.

Ac(Api)$_8$(^{L}Leu)OBn (= **Api$_8$-^{L}Leu**)

By following the same procedure used for the preparation of Ac(^{L}Leu)(Api)$_{16}$OBn, Ac(^{H}Api)$_8$(^{L}Leu)OBn (62 mg, 40 µmol) was converted into Ac(Api)$_8$(^{L}Leu)OBn as a white solid (50 mg, 40 µmol, quant). ^{1}H NMR (500.16 MHz; CD_3OD; 25 °C): δ 7.40–7.20 (m, 5H), 5.11 (m, 2H), 4.46 (m, 1H), 3.34–2.43 (m, 32H), 2.39–1.53 (m, 38H), 0.90 (m, 6H) ppm; HR-MALDI-TOF-MS calcd. for $C_{63}H_{101}N_{17}O_{11}Na$ ([M + Na]$^+$) 1294.7759, found 1294.7719.

Ac(^{Boc}Api)$_{16}$(^{L}Leu)OBn (= **^{Boc}Api$_{16}$-^{L}Leu**)

To a stirred CH_2Cl_2 solution (1 mL) of Ac(^{Boc}Api)$_8$OH (187 mg, 100 µmol) at 0 °C, were added HOAt (14 mg , 100 µmol), HATU (38 mg, 100 µmol), and TMP (40 µL, 300 µmol), and the mixture was stirred for 3 h at 0 °C. Then, H(^{Boc}Api)$_8$(^{L}Leu)OBn (203 mg, 100 µmol) was added at 0 °C to the reaction mixture. After being stirred at 0 °C for 30 min, the reaction mixture was allowed to warm to rt and stirred for 4 days. Then, the solution was diluted with CH_2Cl_2 (10 mL), washed with aqueous HCl (1 M, 3 × 10 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated to dryness under a reduced pressure at rt. The residue was subjected to silica gel column chromatography eluted with AcOEt/$CHCl_3$ (1:1–2:1, v/v) to afford Ac(^{L}Leu)(^{Boc}Api)$_{16}$OBn as a white solid (136 mg, 35 µmol, 35%). ^{1}H NMR (500.16 MHz; CD_3OD; 50 °C): δ 7.39–7.27 (m, 5H), 5.11 (ABq, 2H, $\Delta\nu_{AB}$ = 12.3 Hz, J_{AB} = 12.6 Hz), 4.50 (m, 1H), 3.98–2.68 (m, 64H), 2.55–1.54 (m, 70H), 1.50–1.39 (m, 144H), 0.93 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.3 Hz, 3H) ppm; ^{13}C NMR (125.77 MHz; CD_3OD/$CDCl_3$ = 2:1 (v/v); 50 °C): δ 177.98, 176.37, 176.29, 176.20, 176.16–175.84, 175.60, 175.10, 173.62, 173.56, 155.93, 155.88, 155.79–155.56, 136.67, 129.15, 129.03, 128.96, 81.42–81.23, 81.21, 81.16, 81.09, 80.95, 80.83, 80.68, 80.10, 80.06, 80.02, 67.49, 59.75, 59.60, 58.45, 58.35, 58.27, 58.20, 58.10, 57.98, 41.59–39.24, 35.47–34.26, 28.96–28.58, 25.14, 23.28, 21.75 ppm; HR-MALDI-TOF-MS calcd. for $C_{191}H_{309}N_{33}O_{51}Na$ ([M + Na]$^+$) 3904.2492, found 3904.2445.

Ac(^{H}Api)$_{16}$(^{L}Leu)OBn (= **^{H}Api$_{16}$-^{L}Leu**)

By following the same procedure used for the preparation of Ac(^{L}Leu)(^{H}Api)$_{16}$OBn, Ac(^{Boc}Api)$_{16}$(^{L}Leu)OBn (105 mg, 27 µmol) was converted into Ac(^{H}Api)$_{16}$(^{L}Leu)OBn as a white hygroscopic solid (77 mg, 27 µmol, quant). ^{1}H NMR (500.16 MHz; CD_3OD; 25 °C): δ 8.81(s, 1H), 8.68 (s, 1H), 8.52 (brs, 1H), 8.50 (s, 1H), 8.35 (s, 1H), 8.30 (s, 1H), 8.21 (brs, 1H), 8.12 (s, 1H), 8.02 (brs, 2H), 7.99 (s, 1H), 7.93 (s, 1H), 7.91 (s, 1H), 7.80–7.73 (m, 3H), 7.68 (s, 1H), 7.52–7.42 (m, 5H), 5.14 (ABq, 2H, $\Delta\nu_{AB}$ = 14.2 Hz, J_{AB} = 11.6 Hz), 3.90 (brs, 1H), 3.72–2.91 (m, 64H), 2.18 (s, 3H), 2.94–2.10 (m, 64H), 1.82–1.67 (m, 3H), 1.02 (d, J = 6.1 Hz, 3H), 0.99 (d, J = 6.1 Hz, 3H) ppm; HR-MALDI-TOF-MS calcd. for $C_{111}H_{181}N_{33}O_{19}Na$ ([M – 16HCl + Na]$^+$) 2303.4104, found 2303.4090.

Ac(Api)$_{16}$(^{L}Leu)OBn (= **Api$_{16}$-^{L}Leu**)

By following the same procedure used for the preparation of Ac(^{L}Leu)(Api)$_{16}$OBn, Ac(^{H}Api)$_{16}$(^{L}Leu)OBn (46 mg, 16 µmol) was converted into Ac(Api)$_{16}$(^{L}Leu)OBn as a white hygroscopic solid (36 mg, 16 µmol, quant). ^{1}H NMR (500.16 MHz; CD_3OD; 25 °C): δ 7.45–7.21 (m, 5H), 5.11 (m, 2H), 4.47 (m, 1H), 3.37–2.48 (m, 64H), 2.45–1.75 (m, 64H), 1.75–1.51 (m, 3H), 0.91 (m, 6H) ppm; HR-MALDI-TOF-MS calcd. for $C_{111}H_{181}N_{33}O_{19}Na$ ([M + Na]$^+$) 2303.4104, found 2303.4078.

Circular Dichroism (CD) Spectroscopy

CD measurements in MeOH were performed by using a quartz cuvette with 1 mm optical path length (Figures 3, 4, and S1–S3). The sample solutions with the following peptide concentrations were used. Also note that essentially no concentration dependence was observed for the CD spectra of all peptides (Figures S2 and S3).

Protonated 8mers	(**^{L}Leu-^{H}Api$_8$** and **^{H}Api$_8$-^{L}Leu**):	300 µM
Free-base 8mers	(**^{L}Leu-Api$_8$** and **Api$_8$-^{L}Leu**):	300 µM
Acylated 8mers	(**^{L}Leu-^{Boc}Api$_8$** and **^{Boc}Api$_8$-^{L}Leu**):	200 µM
Protonated 16mers	(**^{L}Leu-^{H}Api$_{16}$** and **^{H}Api$_{16}$-^{L}Leu**):	200 µM
Free-base 16mers	(**^{L}Leu-Api$_8$** and **Api$_8$-^{L}Leu**):	200 µM
Acylated 16mers	(**^{L}Leu-^{Boc}Api$_{16}$** and **^{Boc}Api$_{16}$-^{L}Leu**):	100 µM

CD measurements in aqueous media were performed by using a quartz cuvette with 10 mm optical path length (Table 1 and Figures 5, S4, and

S5). The sample solutions with the peptide concentration of 30 μm were used.

Mean residue molar ellipticity ($[\theta]_{MRE}$) was calculated from ellipticity (θ) using the following equations:

$$[\theta]_{MRE} = (100 \times \theta)/(C \times L \times m)$$

θ : ellipticity (degree)
C : peptide concentration (mol L^{-1})
L : light path length of cuvette (cm)
m: number of amino acid residues

pH Titration Experiments

Typically, **^{L}Leu-^{H}Api$_{16}$** (4.3 mg, 1.5 μmol) was dissolved in water (50 mL) to afford an aqueous solution with the peptide concentration of 30 μM. The pH value of the aqueous solution was adjusted to 2.0 by adding an aqueous solution of HCl (6.0 M). To the aqueous solution was added an aqueous solution of NaOH (5.4×10^{-n} M, n = 1–3) in a stepwise manner with magnetic stirring. After each step of the NaOH addition, pH of the aqueous solution was measured. Then, a part of the aqueous solution (~3 mL) was taken into a quartz cuvette and subjected to CD measurement. After the CD measurement, the portion taken in the quartz cuvette was combined with the mother liquor and subjected to the next step of the NaOH addition. The pH titration experiment of **^{H}Api$_{16}$-^{L}Leu** was conducted in the same manner.

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Keywords: helix • oligopeptides • chain length effects • chiral induction • dynamic helical peptide

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[28] Deprotection of benzyl esters were performed with 5 wt% Pd/C and H_2 (1 atm) in THF/MeOH (3:1, v/v).

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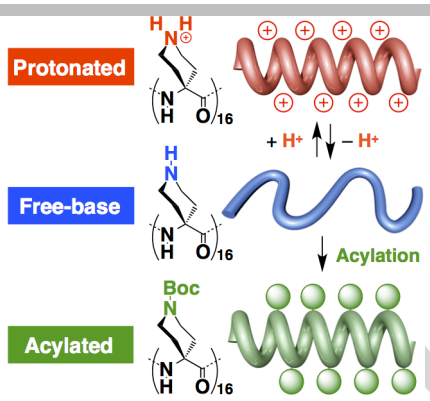
Dynamic Helices: As a new motif for the study of dynamic helices, hexadecamers of 4-aminopiperidine-4-carboxylic acid (Api), bearing L-leucine as a chiral auxiliary at either N- or C-terminus, was developed. Although the Api hexadecamer possesses only one stereogenic center, it showed notably high helicity. The helicity of Api peptides was readily modulated by changing the peptide length, state of the piperidine groups in the Api units, and position of the chiral auxiliary.



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