The Effect of a Peptide Helix Macrodipole on the pK_a of an Asp Side Chain Carboxylate

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Abstract: A study of the effect of a helix dipole on the pK_a of a side chain functional group has been undertaken to determine the magnitude of these electrostatic effects in the absence of interfering influences from a protein matrix. Three helical peptides were prepared: two containing Asp residues at the N- or C-terminus and one with an Asp residue in the middle of the peptide. These peptides have no reactive residues other than the Asp side chain carboxylate group. Circular dichroism confirmed that these peptides adopt helical conformations in aqueous solution over a broad pH range. The pK_a of compound **12**, where the Asp residue is at the N-terminus of the helix, is 3.81 ± 0.31. This is lower than the pK_a of an Asp residue in a short nonhelical model compound (4.09 ± 0.21) and lower than the pK_a values of **23**, where the Asp residue is at the C-terminus of a helix (4.17 ± 0.24), and **19**, where the Asp residue is in the middle of the helix (4.17 ± 0.29). No significant perturbation was observed at the C-terminus of a helix (compound **23**), despite this being the negative pole of the dipole. We believe that this carboxylate is drawn toward the N-terminus by electrostatic attraction to the positive pole of the dipole, resulting in positioning of the carboxylate over the middle of the helix rather than over the C-terminus.

α-Helices are common structural elements in a diverse array of proteins. A substantial permanent dipole moment in these structures results from the near-perfect alignment of amide carbonyl groups with the helix axis.[1,2] Gas phase calculations have suggested that the resulting electrostatic field is roughly equivalent to placement of half of a positive charge at the N-terminus and half of a negative charge at the C-terminus of the helix,[2] although in solution this electric field would be attenuated by solvent dielectric screening.

The electric field of a peptide helix is sufficiently strong, even in fairly short peptide systems, to affect photoinduced electron transfer processes[3] and the absorption spectrum of pendant chromophores.[4] The presence of highly polar helical structures within proteins has lead a number of researchers to study the possibility that the "macrodipole" of helices could perturb the chemistry of nearby functional groups, thereby influencing reaction rates, functional group pK_a values, or substrate binding constants.[5] For example, an α-helix at the active site of triosephosphate isomerase perturbs the pK_a of nearby His residues.[6] His-95, positioned in a buried active site near the N-terminus of an α-helix, had a pK_a of 12, which is 2 units lower than the normal value for a His residue. In addition, His-95 forms a hydrogen bond with the main chain amide of Glu-97, and this may also help in lowering the pK_a. Thus, there are two factors acting together to lower the pK_a of His-95. His-103, located near the C-terminus of an α-helix, had a pK_a of 7.3, which is about 0.6 units higher than the normal value of histidinium residues. However, this residue is located on the outer surface of the enzyme where the helix dipole is partially screened at the surface of the enzyme by solvent. Dielectric screening is lower in a buried active site that is not accessible to bulk water.

A similar study on the ribnuclease barnase revealed an unusual pK_a value for a His residue.[7] A single histidine residue was placed at either the N- or the C-terminus of the two α-helices of barnase by using site-directed mutagenesis. The pK_a of His residues at the C-termini of the helices were, on average, 0.5 units higher than normal, and the pK_a of His residues at the N-termini were lower by 0.8 units, compared to the pK_a of His residues in the denatured protein at low ionic strength.[8] These results are consistent with the view that the helix dipole perturbs these functional groups.

However, other structural effects can cause significant perturbations in the pK_a values of acidic residues. For example, Woodward and co-workers[9] reported that the side chain carboxyl pK_a value of an Asp residue in oxidized *Escherichia coli* thioredoxin is 7.5, which is 3 units higher than the normal pK_a value for a solvated carboxylic side chain.[9] The abnormally high pK_a was attributed to the hydrophobic local environment of this carboxyl group in folded thioredoxin.[10]

It is evident that the pK_a values of residues in proteins are determined by a number of different factors, only one of which is the electric field associated with the helix dipole. Other factors include hydrogen bonding, a hydrophobic environment, the effects of solvation, and other details of the microenvironment surrounding that residue. These factors may reinforce or attenuate each other. For example, an acidic residue located in a hydrophobic cleft would have a higher pK_a than the same residue in a hydrophilic environment. If this residue also happens to be located at the C-terminal of the helix, the pK_a would increase further. If this residue is at the N-terminus of a helix, then the effect of the helix dipole would at least partially cancel the effect of the hydrophobic environment. Given the

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complexities of protein structure, it is difficult (or impossible) to measure the influence of a helix dipole on the pK_a of a residue without measuring an ensemble of all these factors.

The aim of this research is to investigate the effect of a helix macrodipole on the chemistry of a nearby functional group while excluding as many of the other factors as possible that may influence that chemistry. In this paper, we report the preparation of a series of simple helical peptides and the perturbation caused by the electric dipole and these peptides on the pK_a of Asp side chain carboxylates.

Experimental Design

Our goal was to synthesize short helical peptides with an aspartic acid residue at one of three different positions along the helix; at the N-terminus, at the C-terminus, and near the center. If the helix dipole is perturbing the chemistry of the Asp residue,[1,5] then the pK_a of an Asp residue at the N-terminus should be lower than that of the Asp located near the center, which in turn should be lower than that of the Asp at the C-terminus.

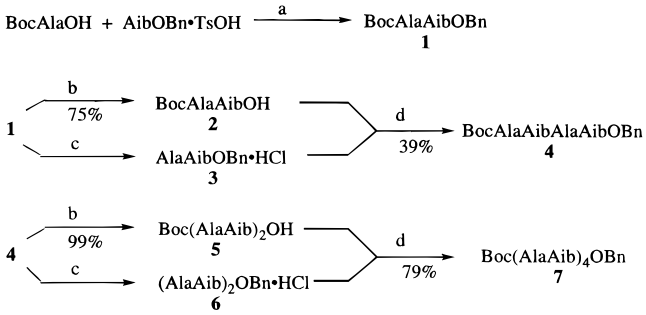
The helices found in enzymes are typically 15−18 amino acid residues long.[11] Calculations by Aqvist and Sitkoff revealed that primary contributions to the helix dipole come from the first and second helical turns,[12,13] thus long helices are not required for a significant dipole. For this study, not only are stable helices needed but also all of the amino acid residues (except the side chain of the aspartic acid) need to have uncharged, unreactive side chains. This creates a system in which there are very few factors that can affect the Asp pK_a, and any perturbation in these pK_a values would most likely be due to the helix dipole.

Simple aliphatic amino acids such as alanine are helix supporting, but β-sheet formation dominates in Ala oligomers.[14] The incorporation of the non-natural amino acid Aib[15] is crucial for helix formation. Aib-containing peptides tend to form helices in a relatively short peptides.[14,16−19] The two methyl groups on the C_α carbon restricts the rotation around N—C_α and C_α—C═O bond.[17] The incorporation of Aib residues into alanine oligomers breaks β-sheet formation, induces helicity,[20] and increases the solubility[14] of the helical oligomers in aqueous and organic solvents.

Results and Discussion

An alternating Ala−Aib sequence was chosen for high helical tendency[16,19,21−25] and for ease of synthesis. Oligopeptides containing Aib have been observed to form 3_{10}-helices,[14,23,26,27] and peptides containing 3−12 Aib residues have been shown to adopt 3_{10}-helical conformations.[28] Structural studies of Aib-containing peptides reveal that Aib has a strong tendency to form 3_{10}-helices in short oligopeptides and β-turns in tri- or tetrapeptides.[19] The strong helix-inducing property of Aib has been documented in numerous crystal structures of short peptides, showing 3_{10}-helices and longer peptides that form mixed 3_{10}-/α-helices.[26] Calculations by Marshall and co-workers suggested that Aib_{10} favors an α-helix conformation over a 3_{10}-helix conformation under aqueous conditions.[29] It has been suggested that 3_{10}-helices can be identified by a weak signal at 222 nm.[30] Unfortunately such an assignment may be deceptive because random coils can also contribute to the circular dichroism (CD) signal, giving a maximum near 222 nm.[31]

Scheme 1[a]



[a] Reaction conditions: (a) NMM, IBCF, THF, −15 °C; (b) H_2, Pd/C, MeOH; (c) HCl(g), CH_2Cl_2; (d) EDC, NMM, BtOH, DMF, 0 °C.

The preparation of **12**, **19**, and **23** was accomplished by a solution phase segment condensation approach, which allowed an Asp residue to be incorporated at the desired position. The synthetic scheme for di- and tetrapeptide building blocks is shown in Scheme 1. Boc-protected alanine was coupled to AibOBn using standard mixed anhydride methods,[32] producing dipeptide **1**. This dipeptide was divided into two batches: one batch was deprotected at the N-terminus and the other batch was deprotected at the C-terminus. The resulting dipeptides were coupled together using EDC to produce tetrapeptide **4**. In a similar fashion, octapeptide **7** was prepared and hydrogenolyzed to produce **8** (Scheme 2).

Methyl ester **9**, produced from benzyl ester **7**, was coupled with a protected Asp residue to produce **11**. Hydrogenolysis of the benzyl ester moiety in **11** to produce **12** was carried out in glacial acetic acid to suppress a rapid side reaction, which leads to succinimide formation.[33]

Dipeptide **2** was coupled to AlaOMe·HCl to generate tripeptide **13** (Scheme 3). After N-deprotection, **13** was coupled to a protected Asp residue to produce **15**. Hydrogenolysis of

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(15) Abbreviations used: Aib, α-aminoisobutyric acid; BtOH, *N*-hydroxybenzotriazole; IBCF, isobutyl chloroformate; NMM, *N*-methylmorpholine; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; Boc, *tert*-butoxycarbonyl. All chiral amino acids are of the L-configuration.
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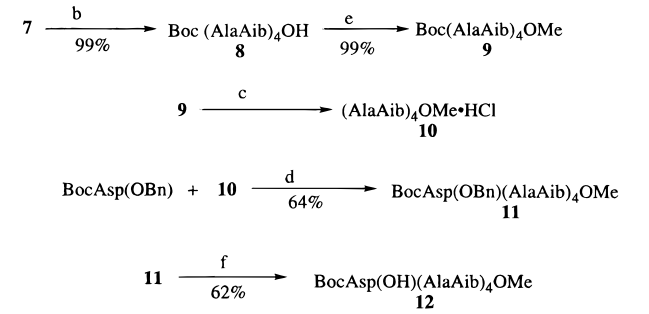
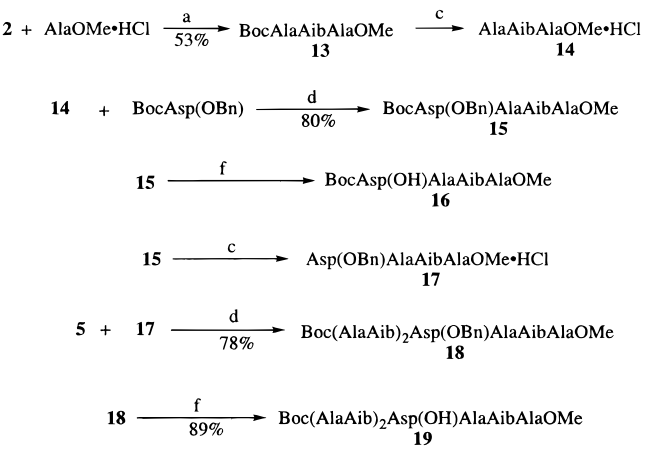
Scheme 2[a]



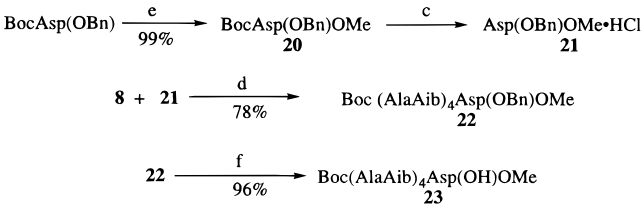
[a] Reaction conditions: (b) H_2, Pd/C, MeOH; (c) HCl(g), CH_2Cl_2; (d) EDC, NMM, BtOH, DMF, 0 °C; (e) CH_2N_2; (f) H_2, Pd/C, AcOH.

Scheme 3[a]



[a] Reaction conditions: (a) NMM, IBCF, THF, −15 °C; (c) HCl(g), CH_2Cl_2; (d) EDC, NMM, BtOH, DMF, 0 °C; (f) H_2, Pd/C, AcOH.

Scheme 4[a]



[a] Reaction conditions: (c) HCl(g), CH_2Cl_2; (d) EDC, NMM, BtOH, DMF, 0 °C; (e) CH_2N_2; (f) H_2, Pd/C, AcOH.

15 produced **16**, which was used as a model compound in NMR titrations. Using the standard protection, deprotection, and coupling steps, **15** was coupled with tetrapeptide **5** to provide **18**. This was hydrogenolyzed in glacial acetic acid to produce **19**.

Asp(OBn)(OMe) (**21**) was prepared from BocAsp(OBn)OH (**20**) and coupled with octapeptide **8** using DCC to generate **22** (Scheme 4). Hydrogenolysis of **22** produced nonapeptide **23**.

Compounds **12**, **19**, and **23** showed spectral data (^1H, ^{13}C NMR) and elemental analyses that were fully consistent with the desired structures. The CD spectra of these compounds showed the characteristic minima of 203 and 218 nm, indicating that these compounds are helical in neutral aqueous solution. We performed CD measurements on **12**, **19** and **23** at pH 2.1, 4.4, and 9.3, spanning the range to be covered in titrations, and it is evident from these measurements that **12**, **19**, and **23** are helical throughout the pH range (Figure 1).

The pK_a values of **12**, **19** and **23** were determined by NMR titration.[34,35] We used ^1H NMR to determine the chemical shift of the side chain CH_2 of the Asp residue at different pH values. The Henderson−Hasselbach equation (eq 1)

$$\mathrm{pH} = \mathrm{p}K + \log [\mathrm{A}^-]/[\mathrm{HA}] \quad (1)$$

can also be written as

$$\mathrm{pH} = \mathrm{p}K + \log \Delta \qquad \text{where } \Delta = (\delta_{max} - \delta)/(\delta - \delta_{min}) \quad (2)$$

where δ is the observed chemical shift in hertz for a resonance with a pH-dependent chemical shift (the Asp side chain CH_2) and δ_{max} and δ_{min} are the maximum and minimum observed chemical shifts, respectively, at the extremes of the pH range (2 to 7.5). A plot of log Δ against pH produces two linear segments that intersect at log $\Delta = 0$ and a pH that corresponds to the pK_a of the acid.

Each compound was titrated three times, using freshly prepared samples each time. The data points for the three runs of the same compound were plotted together.

The pK_a of compound **12**, where Asp residue is at the N-terminus of the helix, is 3.81 ± 0.31 (Figure 2). This is lower than the pK_a of **16**, a short nonhelical peptide with Asp residue at N-terminus of the chain, which is 4.09 ± 0.21. The pK_a of **23**, where the Asp residue is at the C-terminus of the helix, is 4.17 ± 0.24 (Figure 4), and the pK_a of **19**, where the Asp residue is in the middle of the helix, is 4.17 ± 0.29 (Figure 3). Analysis of variance reveals that the measured pK_a for **12** is significantly different (at the 95% confidence level) from the other pK_a values.

These results show that there is perturbation of the pK_a of an Asp residue at the N-terminus of a helix (as in **12**) but that no significant perturbation occurs in compounds where the Asp carboxylate is located either in the middle (**19**) or at the C-terminus (**23**) of the helix. The action of an electric dipole should lower the pK_a of a residue at the N-terminus but *raise* the pK_a of a residue at the C-terminus. However, we observe very similar pK_a values for **19** and **23**. We believe the reason is that the carboxylate group of the Asp residue in **12** is oriented so that it is close to the N-terminus of the helix, since N-terminus is the positive pole of the dipole. However, in **23** the carboxylate group is also drawn toward the N-terminus; therefore, the C-terminal Asp side chain in **23** is not positioned over the C-terminus (negative pole) of the helix but over the side of the helix in a position like that of the carboxylate in **19**. A similar phenomenon has been reported by Matthews.[36] This leads to **19** and **23** showing identical pK_a values that are similar to the pK_a of nonhelical **16**. The orientations of Asp carboxylate groups in these three compounds is shown schematically in Figure 5.

Conclusions

We have prepared a series of helical peptides that bear a single Asp residue located at different positions in the different peptides. Titration of these peptides reveals that the pK_a of an Asp residue located at the N-terminus of a helix is 0.31 units lower than the pK_a of Asp residues located elsewhere, consistent with a perturbation induced by the helix macrodipole. The perturbation we observe in this simple system is smaller than perturbations reported for amino acid residues in some proteins and corresponds to a stabilization of −0.409 kcal/mol.

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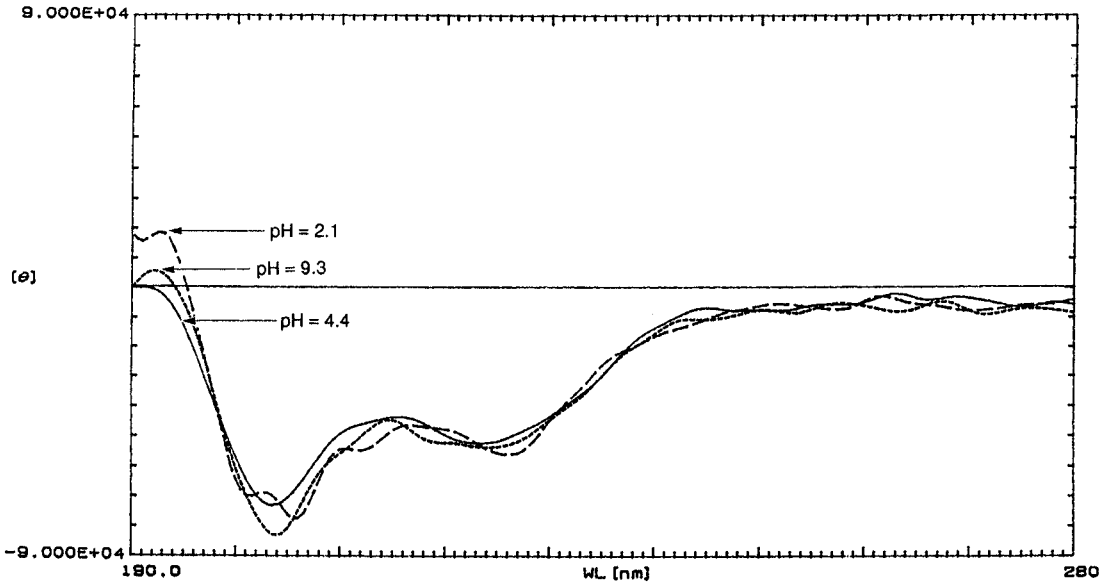

Figure 1. Circular dichroism spectra of nonapeptide **12** (875 μM) in D_2O at different pH values and constant ionic strength. Data were obtained on JASCO 700 instrument and expressed in terms of molar ellipticity.

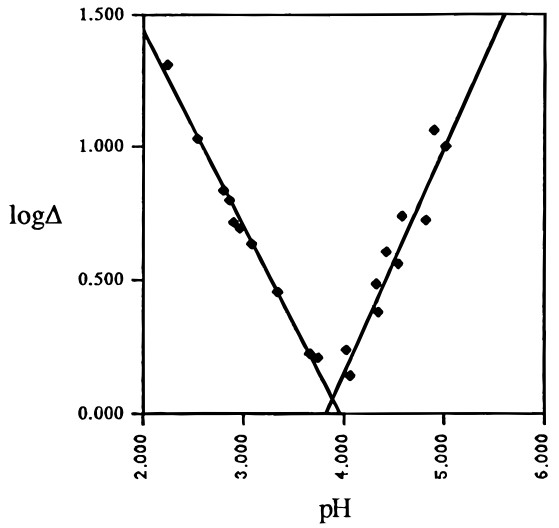

Figure 2. NMR titration of BocAsp(OH)(AlaAib)$_4$OMe (**12**) in D_2O.

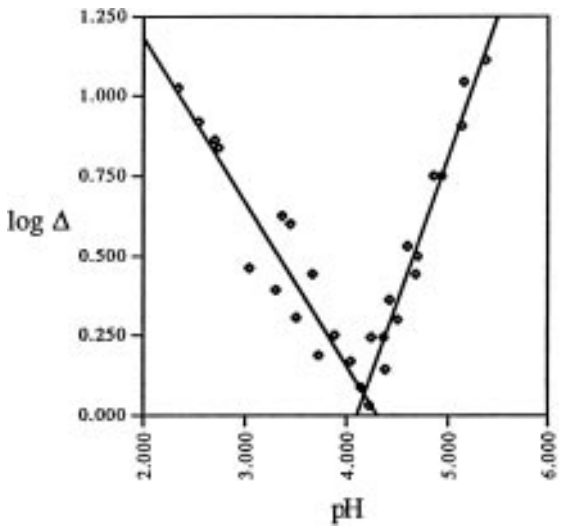

Figure 3. NMR titration of Boc(AlaAib)$_2$Asp(OH)AlaAibAlaOMe (**19**) in D_2O.

The free carboxylates in **12**, **19**, and **23** are likely to be fully solvated, thus the contribution to the shift in pK_a due to intramolecular hydrogen bonding in **12** is probably small, consistent with the fairly normal pK_a for **16** which also has the opportunity for intramolecular hydrogen bonding. It is also unlikely that the differences in solvation between these three compounds are sufficient to account for the observed shift in pK_a. The perturbation we observe in the pK_a of the Asp side chain in **12** (0.3 units) is lower than many of the reports of helix dipole-induced pK_a shifts in proteins. The dipoles of the isolated helices studied here are fully solvated, and therefore there is maximum chance for dielectric screening from the polar, ionic medium. The influence of helices within relatively hydrophobic active sites, where bulk water is (partially or largely) excluded, should be more pronounced.

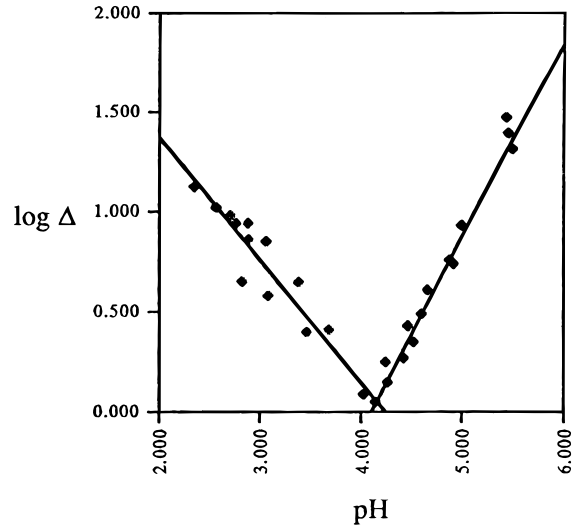

Figure 4. NMR titration of Boc(AlaAib)$_4$Asp(OH)OMe (**23**) in D_2O.

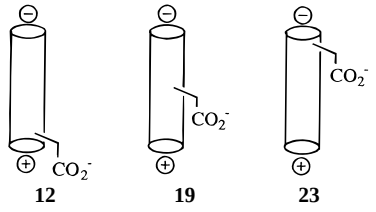

Figure 5. Schematic representation of the orientation of the carboxylate groups in **12**, **19**, and **23**.

Experimental Section

General Procedures. Commercial solvents and reagents were used without further purification, except for tetrahydrofuran which was distilled under nitrogen from CaH_2 and then distilled from sodium benzophenone ketyl. Flash column chromatography was performed under slight positive air pressure on Baker silica gel (40 μm average particle diameter); thin layer chromatography (TLC) was performed on precoated silica gel 60 plates (0.2 mm thickness). Melting points are uncorrected.

Sample Preparation. The samples for NMR titrations were prepared as aqueous solutions in D_2O at different pH values with a constant peptide concentration of 0.875 mM. The ionic strength was kept constant throughout the experiment at 0.875 mM using various amounts of tetramethylammonium bromide, tetramethylammonium hydroxide, and deuterated acetic acid. All proton NMR spectra were measured on a VXR-400 instrument in 5 mm sample tubes. A capillary tube containing 2% 1,4-dioxane and 98% deuterium oxide was used as an external reference. All chemical shift values were assigned relative to the dioxane resonance at 3.7 ppm. Circular dichroism measurements were carried out at ambient temperature on JASCO 710 instrument in a cell with 0.01 cm path length. Samples from NMR titrations at different pH values, prepared in D_2O with a peptide concentration of 875 μM (1750 μM at neutral pH), were used for CD spectroscopy.

AibOBn·TsOH.[37] To a 500 mL round-bottomed flask were added 25.8 g (0.25 mol) of Aib, 48.5 g (0.255 mol) of *p*-toluenesulfonic acid, 100 mL of benzyl alcohol, and 50 mL of benzene. The mixture was heated at reflux, with water being removed by Dean−Stark distillation. When the water ceased to distill (after the collection of about 14.5 mL), the reaction mixture was cooled to room temperature. The reaction mixture solidified upon standing and was then recrystallized with methanol/ether to provide the product (80 g, 87%) as a white crystalline solid, mp 150−152 °C (lit.[37] 150−152 °C): ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.48 (s, 6H), 2.29 (s, 3H), 5.26 (s, 2H), 7.09−7.50 (m, 9H); ^{13}C NMR (50 MHz, DMSO) δ 20.83, 23.35, 55.93, 67.34, 125.52, 127.94, 128.18, 128.39, 128.58, 135.29, 137.92, 145.37, 171.38; IR (KBr) 2990, 1751, 1654, 1617, 1545, 1499, 1466, 1397, 1327, 1182, 1126, 1034, 1010, 812, 765, 734, 696, 683, 604, 566, 538 cm^{-1}.

BocAlaAibOH (2).[24] BocAlaOH (3.784 g, 20.0 mmol) was dissolved in 200 mL of dry THF. The solution was chilled to −15 °C in a ice−salt bath. NMM (4.6 mL, 20.0 mmol) was added dropwise, followed by dropwise addition of IBCF (2.6 mL, 20.0 mmol), and a white precipitate formed. To this suspension was added solid AibOBn·TsOH (7.3 g, 20.0 mmol). The suspension was kept stirring overnight at room temperature. The reaction mixture was concentrated, and the residue was dissolved in ethyl acetate. The organic layer was then washed with H_2O, 10% HCl solution, and saturated solutions of $NaHCO_3$ and NaCl. The solution was dried over Na_2SO_4 and then concentrated in vacuo. The residue (**1**, yellow oil) was dissolved in 100 mL of EtOH. To this reaction mixture were added 1.2 g of Pd/C as catalyst. The reaction was stirred vigorously overnight under 1 atm of H_2. The reaction mixture was then filtered to remove the catalyst and concentrated in vacuo to obtain the crude product. Recrystallization from EtOAc/hexane provided **2** (4.13 g, 75% calcd from BocAlaOH) as a white solid, mp 174−176 °C (lit. 178 °C,[38] 170 °C[27]): ^{1}H NMR (200 MHz, methanol-d_4) δ 1.27 (d, J = 7.16 Hz, 3H), 1.44 (s, 9H), 1.47 (s, 3H), 1.50 (s, 3H), 4.04 (m, 1H), 8.03 (s, 1H); ^{13}C NMR (50 MHz, methanol-d_4) δ 18.37, 25.03, 25.27, 28.67, 51.38, 56.96, 80.57, 157.48, 174.88, 177.71; IR (KBr) 3388, 3380, 2992, 2540, 1693, 1627, 1538, 1498, 1463, 1368, 1295, 1246, 1209, 1175, 1074, 1045, 1028, 1002, 956, 916, 855, 792, 761, 682, 574, 511, 458 cm^{-1}.

Boc(AlaAib)$_2$OBn (4). In a 250 mL round-bottomed flask were added 4.56 g (16.64 mmol) of BocAlaAibOH (**2**) and AlaAibOBn·HCl (**3**, prepared by dissolving 6.99 g (20 mmol) of BocAlaAibOBn (**1**, prepared as above) in dioxane, bubbling HCl gas through the solution, and evaporation to dryness). To this reaction mixture was added a 150 mL of DMF. The reaction mixture was stirred, and 2.19 mL (19.9 mmol) of NMM was added followed by addition of 3.31 g (21.63 mmol) of BtOH and 3.83 g (19.96 mmol) of EDC, respectively. The reaction mixture was stirred at room temperature for 36 h. The reaction mixture was concentrated and extracted with ethyl acetate. The organic layer was washed twice with 10% HCl solution, once with water, twice with saturated $NaHCO_3$, and once with saturated brine. The organic layer was dried over Na_2SO_4 and then concentrated in vacuo. The product was purified by trituration with diethyl ether to obtain **4** (3.39 g, 39%) as a white solid, mp 159−160 °C; ^{1}H NMR (200 MHz, acetone-d_6) δ 1.28−1.48 (m, 27H), 3.90 (m, 1H), 4.21 (m, 1H), 5.08 (s, 2H), 6.56 (br s, 1H), 7.21−7.67 (m, 8H); ^{13}C NMR (50 MHz, acetone-d_6) δ 17.19, 17.68, 24.22, 25.15, 25.68, 27.19, 28.57, 50.14, 53.17, 56.25, 57.31, 66.66, 80.31, 128.45, 128.55, 129.09, 137.76, 157.44, 172.59, 174.20, 174.62, 174.71; IR (KBr) 3328, 2972, 1725, 1686, 1658, 1525, 1453, 1375, 1358, 1303, 1247, 1158, 1066, 1020, 753, 697, 586 cm^{-1}; FAB MS *m/e* 653.1 (M + Cs)$^+$. Anal. Calcd for $C_{26}H_{40}N_4O_7$: C, 59.98; H, 7.74; N, 10.76. Found: C, 59.82; H, 7.70; N, 10.69.

Boc(AlaAib)$_2$OH (5). Boc(AlaAib)$_2$OBn (0.78 g, 1.5 mmol) was dissolved in 100 mL of methanol. To this reaction mixture were added 0.1 g of activated charcoal and 0.157 g of Pd/C. The reaction was stirred overnight under 1 atm of H_2. The suspension was then filtered to remove the catalyst and concentrated in vacuo to provide **5**, 0.64 g (99%), as a white solid, mp 136−137 °C (foams at 90−95 °C); ^{1}H NMR (400 MHz, methanol-d_4) δ 1.28−1.49 (m, 27H), 3.92 (q, J = 6.94 Hz, 1H), 4.23 (q, J = 7.51 Hz, 1H); ^{13}C NMR (100 MHz, methanol-d_4) δ 17.35, 17.61, 24.90, 25.06, 25.50, 26.11, 28.73, 50.90, 52.87, 57.24, 57.68, 80.99, 158.26, 174.16, 175.88, 176.66, 178.18; IR (KBr) 3308, 2983, 2933, 1655, 1528, 1456, 1367, 1300, 1252, 1167, 1072, 596 cm^{-1}; FAB MS *m/e* 453.0 (M + Na)$^+$. Anal. Calcd for $C_{19}H_{34}N_4O_7 \cdot {}^1/_2H_2O$: C, 51.92; H, 8.03; N, 12.75. Found: C, 52.31; H, 8.01; N, 12.73.

Boc(AlaAib)$_4$OBn (7). In a 250 mL round-bottomed flask were added 2.57 g (5.97 mmol) of Boc(AlaAib)$_2$OH (**5**) and (AlaAib)$_2$OBn·HCl (**6**, prepared by dissolving 3.6 g, 6.92 mmol of Boc(AlaAib)$_2$OBn in dioxane and bubbling HCl gas through the solution). To this reaction mixture was added 150 mL of DMF. The reaction mixture was stirred, and 2.19 mL (19.9 mmol) of NMM was added followed by addition of 1.21 g (7.9 mmol) of BtOH and 1.37 g (7.16 mmol) of EDC, respectively. The reaction mixture was kept stirring at room temperature for 36 h. The reaction mixture was concentrated and extracted with ethyl acetate. Some of the compound precipitated and was collected by filtration. The organic layer was washed twice with 10% HCl solution, once with water, twice with saturated $NaHCO_3$, and once with saturated brine, successively. The organic layer was dried over Na_2SO_4 and then concentrated in vacuo. The product (including the filtered residue) was purified by trituration with diethyl ether, producing **7** (3.90 g, 79%) as a white solid, mp 231−233 °C; ^{1}H NMR (200 MHz, acetone-d_6) δ 1.32−1.53 (m, 45H), 3.80−4.28 (m, 4H), 5.09 (s, 2H), 6.76 (br s, 1H), 7.21−8.14 (m, 12H); ^{13}C NMR (100 MHz, methanol-d_4) δ 16.95, 17.06, 17.12, 17.52, 23.73, 23.85, 24.18, 24.87, 25.74, 27.08, 27.11, 27.22, 28.80, 51.07, 53.14, 53.53, 53.89, 57.19, 57.51, 57.75, 57.95, 67.79, 81.35, 129.05, 129.11, 129.47, 137.61, 158.78, 174.61, 175.76, 175.98, 176.09, 176.49, 177.09, 178.11, 178.25; IR (KBr) 3300, 2978, 2933, 1733, 1656, 1533, 1450, 1383, 1300, 1256, 1222, 1161, 1050, 1028, 850, 694 cm^{-1}; FAB MS *m/e* 965.2 (M + Cs)$^+$. Anal. Calcd for $C_{40}H_{64}N_8O_{11} \cdot {}^1/_2H_2O$: C, 57.06; H, 7.78; N, 13.31. Found: C, 57.00; H, 7.76; N, 13.25.

Boc(AlaAib)$_4$OH (8). Boc(AlaAib)$_4$OBn (**7**, 2.0 g, 2.4 mmol) was dissolved in 100 mL of methanol. To this reaction mixture were added 0.5 g of activated charcoal and 0.4 g of Pd/C, and the suspension was stirred overnight under 1 atm of H_2. The mixture was filtered and then concentrated in vacuo to produce **8** in pure form, 1.77 g (99%), as a white solid, mp 226−228 °C (foams at 145−150 °C): ^{1}H NMR (400 MHz, methanol-d_4) δ 1.32−1.52 (m, 45H), 3.87−4.26 (m, 4H); ^{13}C NMR (100 MHz, methanol-d_4) δ 16.96, 16.99, 17.08, 17.44, 23.75, 23.89, 24.58, 25.08, 25.52, 26.64, 27.03, 27.11, 28.76, 51.03, 52.78, 53.48, 53.77, 57.34, 57.50, 57.78, 57.96, 158.72, 174.34, 175.65 (two superimposed lines), 176.04, 176.37, 177.09, 178.02, 178.18; IR (KBr) 3315, 2984, 2940, 1655, 1528, 1458, 1367, 1304, 1252, 1166, 1073, 1023, 586 cm^{-1}; FAB MS *m/e* 765.5 (MNa)$^+$. Anal. Calcd for $C_{33}H_{58}N_8O_{11}$: C, 53.35; H, 7.89; N, 15.08. Found: C, 52.98; H, 7.90; N, 14.95.

(37) Balasubramanian, T. M.; Kendrick, N. C. E.; Taylor, M.; Marshall, G. R.; Hall, J. E.; Vodyanoy, I.; Reusser, F. *J. Am. Chem. Soc.* **1981**, *103*, 6127−6132.

Boc(AlaAib)$_4$OMe (9). In a 250 mL round-bottomed flask was added 0.743 g (1 mmol) of **8** dissolved in 100 mL of methylene chloride. To this flask was added 3 equiv of diazomethane dissolved in ether. After 30 min, excess of diazomethane was quenched with acetic acid. The solvent was evaporated to obtain the title compound **9**, 0.750 g (99%), as a white solid, mp 226 °C: ^{1}H NMR (200 MHz, acetone-d_6) δ 1.32−1.52 (m, 45H), 3.57 (s, 3H), 3.89−4.21 (m, 4H), 6.74 (br s, 1H), 7.34−8.04 (m, 7H); ^{13}C NMR (100 MHz, acetone-d_6 at 35 °C) δ 16.89, 16.93, 17.05, 17.53, 23.50, 23.54, 24.08, 25.32, 25.61, 27.41, 27.45, 27.59, 28.63, 50.15, 51.88, 53.18, 53.59, 53.78, 56.17, 57.21, 57.31, 57.49, 80.78, 157.97, 172.79, 174.70, 174.90, 175.34, 175.38, 175.54, 177.08, 177.21; IR (KBr) 3308, 2984, 2933, 1734, 1654, 1540, 1458, 1385, 1303, 1256, 1222, 1159, 618 cm^{-1}; HRMS (MALDI) *m/e* (M + Na)$^+$ calcd for 779.428, found 779.427; FAB MS *m/e*; (M + Cs)$^+$ calcd for 889.3435, found 889.3432.

BocAsp(OBn)(AlaAib)$_4$OMe (11). BocAsp(OBn)OH (0.127 g, 0.392 mmol) was dissolved in 50 mL of DMF. To this solution were added **10** (prepared by deprotecting **9** (0.3 g, 0.396 mmol)), NMM (47 μL, 0.428 mmol), and BtOH (0.071 g, 0.464 mmol). The solution was stirred at 0 °C in an ice−salt bath. To this solution was added EDC (0.082 g, 0.428 mmol), and the reaction was stirred at 0 °C for 3 h and then at room temperature for 33 h. The reaction mixture was concentrated, and the residue was dissolved in ethyl acetate. The organic layer was then washed twice with 10% HCl solution followed by water, twice with saturated $NaHCO_3$ followed by water, and finally with saturated brine. The solution was dried over Na_2SO_4 and then concentrated in vacuo. The residue was purified using flash column chromatography (silica column) and 3−5% methanol in dichloromethane to obtain 0.22 g (64%) of title compound **11** as a white solid, mp 88 °C (dec); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40−1.59 (m, 45 H), 2.94 (dd, J = 17.87, 5.10 Hz, 1H), 3.00 (dd, J = 17.87, 6.83 Hz, 1H), 3.67 (s, 3H), 3.91−4.02 (m, 3H), 4.32 (m, 1H), 4.42 (dd, J = 6.83, 5.10 Hz, 1H), 5.12 (s, 2H), 5.58 (br s, 1H), 7.20−7.60 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 35 °C) δ 16.53 (two superimposed lines), 16.97, 17.04, 22.92, 23.03 (two superimposed lines), 23.57, 24.94, 25.17, 27.12, 27.24, 28.27, 35.25, 49.71, 51.85, 52.00, 52.47, 52.51, 52.71, 55.82, 56.55, 56.67, 56.91, 67.16, 81.30, 128.13, 128.65, 128.72, 135.07, 156.25, 171.04, 172.82, 172.99, 173.02, 174.13, 174.24, 175.02, 175.40, 176.10, 176.32; IR (KBr) 3310, 2984, 2933, 1735, 1719, 1686, 1654, 1648, 1638, 1560, 1542, 1499, 1458, 1388, 1364, 1300, 1217, 1160, 1053, 755 cm^{-1}; HRMS (MALDI) *m/e* (M + Na)$^+$ calcd 984.502, found 984.503.

BocAsp(OH)(AlaAib)$_4$OMe (12). A round-bottomed flask was charged with 0.23 g (0.239 mmol) of **11** dissolved in 15 mL of acetic acid. To this reaction mixture was added 0.104 g of Pd/C. The reaction was stirred at room temperature overnight under positive hydrogen pressure. The reaction mixture was then filtered through Celite to remove the catalyst. The solvent was removed in vacuo, and the residue was dissolved in methylene chloride and triturated with ether to obtain 0.13 g (62 %) of the title compound **12** as a white solid, mp 122−124 °C (dec); ^{1}H NMR (400 MHz, methanol-d_4) δ 1.39−1.54 (m, 45H), 2.75 (dd, J = 17.04, 4.09 Hz, 1H), 2.91 (dd, J = 17.04, 8.34 Hz, 1H), 3.65 (s, 3H), 4.02 (m, 3H), 4.18 (q, J = 7.5 Hz, 1H), 4.41 (dd, J = 4.09, 8.34 Hz, 1H); ^{13}C NMR (100 MHz, methanol-d_4, at 35 °C) δ 16.84, 16.86, 16.92, 17.42, 23.52, 23.61, 24.07, 25.05, 25.48, 26.91, 26.98, 27.06, 28.70, 36.98, 51.13, 52.19, 52.60, 53.33, 53.51, 53.60, 57.15, 57.50, 57.55, 57.91, 81.17, 157.89, 174.67 (two superimposed lines), 174.81, 175.67, 176.11, 176.34, 176.59, 177.17, 177.99, 178.16; IR (KBr) 3333, 2989, 2933, 2367, 1733, 1683, 1672, 1666, 1529, 1456, 1389, 1300, 1222, 1194, 1167, 668 cm^{-1}; FAB MS (positive) *m/e* 894.5 (M + Na)$^+$; FAB MS (negative) *m/e* 870 (M − H). Anal. Calcd for $C_{38}H_{65}N_9O_{14}$·CH_3COOH: C, 51.55; H, 7.46; N, 13.52. Found: C, 51.95; H, 7.44; N, 13.43.

BocAlaAibAlaOMe (13).[38] BocAlaAibOH (**2**, 4.13 g, 15.07 mmol) was dissolved in 150 mL of dry THF. The solution was chilled to −15 °C in a ice−salt bath. NMM (3.475 mL, 31.65 mmol) was added dropwise followed by dropwise addition of IBCF (2.15 mL, 16.58 mmol). A white precipitate formed. To this suspension was added solid AlaOMe·HCl (2.314 g, 16.58 mmol). The suspension was stirred overnight at room temperature. The reaction mixture was concentrated, and the residue was dissolved in ethyl acetate. The organic layer was then washed with H_2O, 10% HCl solution, saturated $NaHCO_3$, and saturated brine. The solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was dissolved in CH_2Cl_2/CH_3OH and recrystallized with hot petroleum ether to obtain the title compound **13**, 2.89 g (53%), as a white crystalline solid, mp 178−79 °C: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.365 (d, J = 7.2 Hz, 3H), 1.41 (d, J = 7.4 Hz, 3H), 1.45 (s, 9H), 1.54 (s, 3H), 1.56 (s, 3H), 3.74 (s, 3H), 4.04 (m, 1H), 4.54 (m, 1H), 4.95 (br s, 1H), 6.59 (s, 1H), 7.07 (d, J = 6.4 Hz, 1H); ^{13}C NMR (50 MHz, methanol-d_4) δ 17.37, 17.56, 24.23, 26.35, 28.67, 49.70, 51.80, 52.65, 57.64, 80.67, 158.21, 174.97, 175.67, 177.01; IR (KBr) 3588, 3400, 3308, 2984, 2938, 2353, 1738, 1701, 1684, 1676, 1646, 1538, 1453, 1376, 1246, 1169, 1061, 1007 cm^{-1}; MS *m/e* 359 (M + H)$^+$.

BocAsp(OBn)AlaAibAlaOMe (15). In a 250 mL round-bottomed flask were added AlaAibAlaOMe·HCl (**14**, HCl salt was prepared by deprotecting 1.306 g (3.634 mmol) of BocAlaAibAlaOMe (**13**) with HCl gas in methylene chloride) and 1.175 g (3.634 mmol) of BocAsp(OBn) dissolved in 60 mL of DMF and 25 mL of methylene chloride. The reaction mixture was stirred and cooled to 0 °C in an ice−salt bath. To this solution were added 0.589 g (4.361 mmol) of BtOH, 1 mL (9.11 mmol) of NMM, and finally 0.769 g (4 mmol) of EDC. The reaction was stirred at 0 °C for 3 h and then at room temperature for 33 h. The reaction mixture was concentrated in vacuo and then dissolved in ethyl acetate. The organic layer was washed twice with 10% HCl solution, once with water, twice with saturated $NaHCO_3$, water, and finally with saturated brine. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude semisolid was then triturated with anhydrous diethyl ether, and the white solid product was filtered and dried to provide **15**, 1.62 g (80%), mp 126 °C: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36−1.55 (m, 21H), 2.75−3.08 (m, 2H), 3.71 (s, 3H), 4.22 (m, 1H), 4.51 (m, 2H), 5.13 (s, 2H), 5.52 (d, J = 7.7 Hz, 1H), 6.70 (s, 1H), 6.94 (d, J = 6.52 Hz, 1H), 7.02 (d, J = 7.04 Hz, 1H), 7.35 (s, 5H); ^{13}C NMR (50 MHz, acetone-d_6) δ 17.40, 17.63, 24.47, 26.58, 28.46, 36.69, 48.94, 50.77, 52.08 (two superimposed lines) 57.33, 66.88, 79.98, 128.80 (two superimposed lines), 129.21, 137.09, 156.39, 171.56, 171.94, 172.07, 173.88, 174.56; IR (KBr) 3362, 3263, 3061, 2984, 2935, 2363, 2344, 1743, 1718, 1700, 1685, 1670, 1654, 1636, 1618, 1560, 1541, 1534, 1508, 1498, 1458, 1388, 1367, 1309, 1251, 1216, 1174, 1050, 965, 868, 750, 700 cm^{-1}; FAB MS *m/e* 565.3 (M + H)$^+$, 587.2 (M + Na)$^+$. Anal. Calcd for $C_{27}H_{40}N_4O_9$: C, 57.43; H, 7.14; N, 9.92. Found: C, 57.51; H, 7.13; N, 9.97.

BocAsp(OH)AlaAibAlaOMe (16). In a 100 mL round-bottomed flask was dissolved 0.125 g (0.221 mm) of **15** in 25 mL of acetic acid. To this reaction mixture was added 0.097 g of Pd/C. The reaction mixture was stirred overnight at room temperature under the positive hydrogen pressure. The reaction mixture was filtered through Celite to remove the catalyst, and the solvent was evaporated in vacuo. The residue was dissolved in dichloromethane and precipitated with hexane to obtain 0.043 g (41%) of the title compound **16** as a white solid, mp 124−126 °C (foams at 74 °C): ^{1}H NMR (200 MHz, acetone-d_6) δ 1.30−1.44 (m, 21H), 2.81 (br s, 2H), 3.64 (s, 3H), 4.14−4.45 (m, 3H), 6.41 (s, 1H), 7.35 (d, J = 6.60 Hz, 1H), 7.44 (s, 1H), 7.80 (br s, 1H); ^{13}C NMR (100 MHz, acetone-d_6 at 35 °C) δ 17.49, 17.74, 24.80, 26.47, 28.61, 36.87, 49.03, 50.85, 52.15 (two superimposed lines), 57.44, 80.05, 156.51, 172.31, 172.36, 174.05 (two superimposed lines), 174.59; IR (KBr) 3318, 2983, 1661, 1528, 1455, 1370, 1168, 1055 cm^{-1}; FAB MS (positive) *m/e* 497.2 (M + Na)$^+$.

Boc(AlaAib)$_2$Asp(OBn)AlaAibAlaOMe (18). Boc(AlaAib)$_2$OH (**5**, 0.35 g, 0.813 mmol) was dissolved in 50 mL of DMF. To this solution were added Asp(OBn)AlaAibAlaOMe·HCl (**17**, prepared by deprotecting **15** (0.505 g, 0.894 mmol)), NMM (200 μL, 1.82 mmol), and BtOH (0.162 g, 1.2 mmol). The solution was stirred at 0 °C in an ice−salt bath. To this solution was added EDC (0.187 g, 0.975 mmol), and the reaction was stirred at 0 °C for 3 h and then at room temperature for 33 h. The reaction mixture was concentrated, and the residue was dissolved in ethyl acetate. The organic layer was then washed twice with 10% HCl solution followed by water, twice with saturated $NaHCO_3$ followed by water, and finally with saturated brine. The solution was dried over Na_2SO_4 and then concentrated in vacuo. The residue was purified using flash column chromatography on silica gel using 1−3%

(38) Oekonomopulos, R.; Jung, G. *Liebigs Ann. Chem.* **1979**, 1151−1172.

methanol in dichloromethane. It was then recrystallized with ethyl acetate/hexane to obtain 0.554 g (78 %) of the title compound **18** as a white crystalline solid, mp 94 °C (dec): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34−1.58 (m, 39H), 2.94−3.16 (m, 2H), 3.65 (s, 3H), 3.85−3.96 (m, 2H), 4.42−4.58 (m, 2H), 4.66−4.72 (m, 1H), 5.05 (d, J = 12.33 Hz, 1H), 5.14 (d, J = 12.34 Hz, 1H), 5.88 (br s, 1H), 6.94−7.81 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.18, 16.80, 17.05, 17.57, 22.82, 23.00, 24.08, 26.43, 26.91, 27.53, 28.22, 35.61, 48.02, 49.16, 51.89, 51.97, 52.65, 53.22, 56.33, 56.95 (two lines), 66.51, 81.50, 128.15, 128.19, 128.49, 135.63, 157.04, 170.17, 170.92, 171.89, 173.39, 173.76, 174.81, 174.97, 175.88, 176.30; IR (KBr) 3310, 2984, 2939, 1742, 1686, 1634, 1542, 1458, 1387, 1365, 1303, 1252, 1168, 1057, 753, 698, 505 cm^{-1}; HRMS (MALDI) *m/e* $(M + Na)^+$ calcd 899.449, found 899.449.

Boc(AlaAib)$_2$Asp(OH)AlaAibAlaOMe (19). In a 100 mL round-bottomed flask was dissolved 0.2 g (0.228 mm) of **18** in 30 mL of acetic acid. To this reaction was added 0.089 g of Pd/C. The reaction mixture was stirred overnight at room temperature under the positive hydrogen pressure. The reaction mixture was filtered through Celite to remove the catalyst, and the solvent was evaporated in vacuo. The residue was dissolved in dichloromethane and precipitated with ether to obtain 0.16 g (89%) of the title compound **19** as a white solid, mp 162−164 °C (dec): ^{1}H NMR (400 MHz, methanol-d_4) δ 1.31−1.55 (m, 39H), 2.84 (dd, J = 18.54, 10.66 Hz, 1H), 2.91 (dd, J = 18.54, 3.18 Hz, 1H), 3.68 (s, 3H), 3.85 (q, J = 7.33 Hz, 1H), 4.05 (q, J = 7.32 Hz, 1H), 4.23 (q, J = 7.32 Hz, 1H), 4.4 (q, J = 7.33 Hz, 1H), 4.52 (dd, J = 10.66, 3.18 Hz, 1H, almost embedded in water peak); ^{13}C NMR (100 MHz, methanol-d_4 at 35 °C) δ 16.97 (two superimposed lines), 17.09, 17.57, 23.99, 24.06, 24.60, 26.53, 26.76, 26.94, 28.76, 36.05, 51.20 (two superimposed lines), 52.60, 53.03, 53.18, 53.81, 57.55, 57.98, 58.00, 81.38, 158.77, 173.56 (two superimposed lines), 174.17, 174.59, 176.32, 176.48, 176.71, 177.95, 178.00; IR (KBr) 3309, 2985, 2362, 1734, 1700, 1687, 1654, 1648, 1559, 1581, 1522, 1538, 1458, 1387, 1303, 1253, 945, 668 cm^{-1}; FAB MS (negative) *m/e* 785 $(M - H)^+$; FAB MS (positive) *m/e* 809 $(M + Na)^+$. Anal. Calcd for $C_{34}H_{58}N_8O_{13}\cdot CH_3COOH$: C, 51.05; H, 7.38; N, 13.23. Found: C, 50.84; H, 7.31; N, 13.37.

BocAsp(OBn)OMe (20). In a 250 mL round-bottomed flask was added 1 g (3.093 mmol) of BocAsp(OBn)OH dissolved in 100 mL of ether. To this reaction flask was added the etheral solution of diazomethane until the yellow color persisted. The reaction mixture was kept at room temperature for 30 min. It was then concentrated in vacuo, and the product was recrystallized with hexane to obtain the title compound **20**, 1.032 g (99%), as a white crystalline solid, mp 62 °C (lit. mp 67−68 °C,[39] 61−62 °C[40]): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.44 (s, 9H), 2.88 (dd, J = 19.05, 4.74 Hz, 1H), 3.03 (dd, J = 19.05, 4.70 Hz, 1H), 3.70 (s, 3H), 4.59 (m, 1H), 5.13 (s, 2H), 5.48 (d, J = 7.24 Hz, 1H), 7.35 (s, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.29, 36.93, 49.99, 52.64, 66.80, 80.17, 128.30, 128.41, 128.59, 135.40, 155.32, 170.73, 171.46.

Boc(AlaAib)$_4$Asp(OBn)OMe (22). Boc(AlaAib)$_4$OH (**8**, 0.743 g, 1 mmol) was dissolved in 50 mL of DMF. To this solution were added Asp(OBn)OMe·HCl (**21**, prepared by dissolving 0.337 g (1 mmol) of **20** in CH_2Cl_2 and bubbling HCl gas through it), NMM (0.219 mL, 2.0 mmol), and BtOH (0.199 g, 1.47 mmol). The solution was stirred at 0 °C in an ice−salt bath. To this solution was added EDC (0.23 g, 1.2 mmol), and the reaction was stirred at 0 °C for 3 h and then at room temperature for 33 h. The reaction mixture was concentrated, and the residue was dissolved in ethyl acetate. The organic layer was then washed twice with 10% HCl solution followed by water, twice with saturated $NaHCO_3$ followed by water, and finally with saturated brine. The solution was dried over Na_2SO_4 and then concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using 1−3% methanol in dichloromethane. It was then recrystallized with ethyl acetate/hexane to obtain 0.76 g (79 %) of the title compound **22** as a white crystalline solid, mp 86 °C (dec): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39−1.58 (m, 45 H), 2.83 (dd, J = 16.31, 8.26 Hz, 1H), 3.00 (dd, J = 16.31, 9.53 Hz, 1H), 3.63 (s, 3H), 3.87−4.02 (m, 3H), 4.31 (m, 1H), 5.00 (dd, J = 8.26, 9.53 Hz, 1H), 5.13 (s, 2H), 5.62 (br s, 1H), 6.86 (s, 1H), 7.26−7.80 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, at 35 °C) δ 16.51, 16.53, 16.86, 16.96, 22.89 (superimposed lines), 23.10, 24.81, 25.78, 26.98, 27.04, 27.70, 28.24, 36.59, 48.88, 49.95, 52.21, 52.58, 52.81, 53.27, 56.32, 56.59, 56.82, 56.84, 66.44, 81.49, 128.06, 128.23, 128.41, 135.84, 157.04, 170.30, 171.64, 172.49, 173.87, 174.15, 174.61, 175.29, 175.45, 176.00, 177.00; IR (KBr) 3314, 2985, 2939, 1736, 1654, 1542, 1522, 1458, 1385, 1364, 1300, 1256, 1189, 1166, 1053, 1022, 852, 754, 698, 503, cm^{-1}; FAB MS *m/e* 962.5 $(MH)^+$; HRMS (MALDI) *m/e* calcd 984.502 $(M\ Na^+)$, found 984.503.

Boc(AlaAib)$_4$Asp(OH)OMe (23). In a 100 mL round-bottomed flask was dissolved 0.2 g (0.208mm) of **22** in 50 mL of methanol. To this reaction was added 0.05 g of Pd/C. The reaction mixture was stirred overnight at room temperature under a positive hydrogen pressure. The reaction mixture was filtered through Celite, and the solvent was evaporated in vacuo. The product was redissolved in dichloromethane and was precipitated with ether to obtain 0.174 g (96%) of the title compound **23** as a white solid, mp >250 °C: ^{1}H NMR (400 MHz, methanol-d_4) δ 1.32−1.53 (m, 45H), 2.72 (dd, J = 16.96, 5.88 Hz, 1H), 2.87 (dd, J = 16.96, 6.85 Hz, 1H), 3.68 (s, 3H), 3.87 (q, J = 7.16 Hz, 1H), 4.03 (m, 2H), 4.17 (q, J = 7.5 Hz, 1H), 4.79 (m, 1H, almost embedded in water peak); ^{13}C NMR (100 MHz, methanol-d_4 at 35 °C) δ 16.85, 17.04 (two superimposed lines), 17.28, 23.66, 23.76, 23.83, 24.89, 26.15, 26.90, 27.13 (two superimposed lines), 28.76, 36.98, 50.43, 51.47, 52.79, 53.54, 53.59, 53.86, 57.47, 57.68, 57.88, 57.99, 158.79, 172.87 (two superimposed lines), 174.59, 176.09, 176.37, 176.44, 177.02, 177.51, 178.23, 178.48; IR (KBr) 3326, 2985, 2362, 1734, 1700, 1664, 1654, 1647, 1559, 1522, 1457, 1386, 1302, 1122, 998 cm^{-1}; FAB MS (negative) *m/e* 870 $(M - H)$. Anal. Calcd for $C_{38}H_{65}N_9O_{14}$: C, 52.34; H, 7.51; N, 14.46. Found: C, 52.05; H, 7.45; N, 14.26.

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Supporting Information Available: CD spectrum of **12**, **19** and **23** in water and for compounds **19** and **23** in D_2O at different pH values, and the titration of nonhelical model compound **16** are available (4 pages). See any current masthead page for ordering and Internet access instructions.

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