An Unnatural Amino Acid that Mimics a Tripeptide β-Strand and Forms β-Sheetlike Hydrogen-Bonded Dimers

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Abstract: Unnatural amino acid **2** (5-HO_2CCONH-2-MeO-C_6H_3-CONHNH$_2$) duplicates the hydrogen-bonding functionality of one edge of a tripeptide β-strand. It is composed of hydrazine, 5-amino-2-methoxybenzoic acid, and oxalic acid groups and is designated by the three-letter abbreviation "Hao" to reflect these three components. The 2,7-di-*tert*-butylfluorenylmethyloxycarbonyl (Fmoc*)- and *tert*-butyloxycarbonyl (Boc)-protected derivatives of Hao are prepared efficiently and in high yield by the condensation of suitably protected derivatives of hydrazine, 5-amino-2-methoxybenzoic acid, and oxalic acid. Fmoc*-Hao and Boc-Hao behave like typical Fmoc- and Boc-protected amino acids and can be incorporated into peptides by standard solid- and solution-phase peptide synthesis techniques using carbodiimide coupling agents. Hao-containing peptide **9** (*i*-PrCO-Phe-Hao-Val-NHBu) forms a β-sheetlike hydrogen-bonded dimer in $CDCl_3$ and $CD_3OD-CDCl_3$ solutions. Peptides containing Hao and natural amino acids display hydrogen-bonding surfaces that are complementary to the hydrogen-bonding edges of protein β-sheets.

Introduction

Recognition between exposed edges of β-sheets is an important mode of protein−protein interaction.[1] These β-sheet interactions between proteins are, for example, critical in the binding of neuronal nitric oxide synthase inhibitory protein to neuronal nitric oxide synthase,[2] PDZ domains to membrane receptor and ion channel proteins,[3] and Ras oncoproteins to the Raf kinase (Figure 1).[4] Interactions between β-sheet edges are also widely involved in protein dimerization and in peptide and protein aggregation.

The edges of protein β-sheets provide alternating arrays of hydrogen-bond donors and acceptors, with the pattern: donor−acceptor, donor−acceptor, donor−acceptor, etc. In β-sheet interactions between proteins, these edges hydrogen bond together. Chemical decoys that duplicate the hydrogen-bonding edges of protein β-sheets hold promise as inhibitors of protein−protein interactions. Although this promise has not yet been achieved, it has been pursued by several researchers within the past decade. Michne and Schroeder created a bicyclic compound that mimics the hydrogen-bonding pattern of one edge of a peptide β-strand to block a putative β-sheet interaction between lymphocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1).[6] Rebek, Pallai, and coworkers have developed bi- and tricyclic β-strand mimics to inhibit a postulated β-sheet interaction between gp120 and the CD4 receptor.[7] Related studies by Schrader and Kirsten have focused on the development of peptidomimetic compounds that mimic the hydrogen-bonding pattern of one edge of a peptide β-strand and bind peptides through β-sheet interactions.[8]

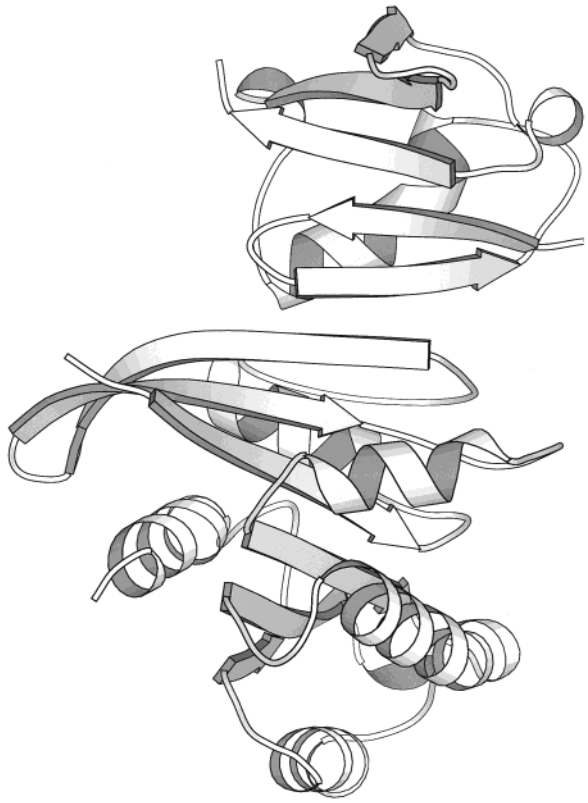

Figure 1. Molscript[5] diagram of the complex between the Ras-binding domain of the *c*-Raf1 kinase (upper) and the Ras analogue, Rap1A (lower). (PDB reference 1gua.[4])

(1) Maitra, S.; Nowick, J. S. β-Sheet Interactions Between Proteins. In *The Amide Linkage: Structural Significance in Chemistry, Biochemistry, and Materials Science*; Greenberg, A., Breneman C. M., Liebman, J. F., Eds.; Wiley: New York, 2000; Chapter 15.

(2) Liang, J.; Jaffrey, S. R.; Guo, W.; Snyder, S. H.; Clardy, J. *Nat. Struct. Biol.* **1999**, *6*, 735−740.

(3) Doyle, D. A.; Lee, A.; Lewis, J.; Kim, E.; Sheng, M.; MacKinnon, R. *Cell* **1996**, *85*, 1067−1076.

(4) Nassar, N.; Horn, G.; Herrmann, C.; Scherrer, A.; McCormick, F.; Wittinghofer, A. *Nature*, **1995**, *375*, 554−560.

(5) Kraulis, P. J. *J. Appl. Crystallogr.* **1991**, *24*, 946−950.

(6) Michne, W. F.; Schroeder, J. D. *Int. J. Pept. Protein Res.* **1996**, *47*, 2−8.

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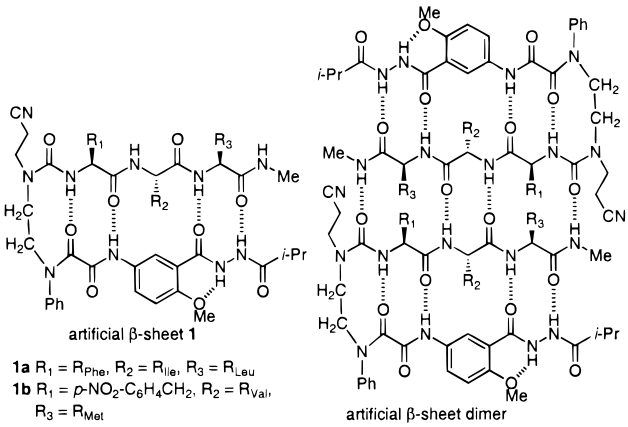
Both hydrogen bonding and other noncovalent interactions contribute to β-sheet interactions between proteins; the hydrogen-bonding interactions between the edges of protein β-sheets are generally accompanied by additional polar and hydrophobic interactions between the amino acid side chains. Contacts with other structures, such as helices, can also be present. Collectively, these important interactions provide strength and specificity to the protein−protein interactions. A variety of systems that mimic and block these interactions have been developed.[9−12]

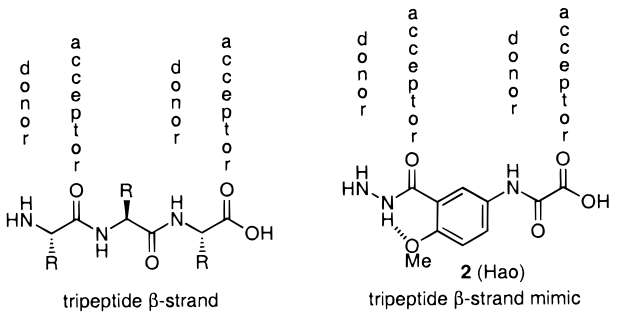
Compounds that mimic the structures and hydrogen-bonding patterns of protein β-sheets but that have not specifically been targeted toward binding proteins have also been reported.[13] In pioneering studies during the late 1980s, Kemp and co-workers described a 2,8-diaminoepindolidione molecular template that mimics the hydrogen-bonding functionality of one edge of a peptide β-strand and have coupled this β-strand mimic to peptides to generate intramolecularly hydrogen-bonded β-sheetlike structures.[14] Our own research group has subsequently developed β-strand mimics based on aminoaromatic derivatives and has combined these β-strand mimics with urea-based turn units and peptide strands to create a variety of β-sheetlike structures, which we have termed *artificial β-sheets*.[13,15,16]

Recently, we reported that combination of a 5-amino-2-methoxybenzoic acid hydrazide β-strand mimic with an oxalamide linker and tripeptides generates artificial β-sheets that form well-defined β-sheet dimers (Scheme 1).[17] Intrigued by the prospect of developing smaller and simpler systems that interact through β-sheet formation, we realized that the 5-amino-2-methoxybenzoic acid hydrazide and oxalamide groups within these molecules could be viewed collectively as an unnatural amino acid that duplicates the hydrogen-bonding functionality of one edge of a tripeptide β-strand. The amino acid, **2**, comprises hydrazine, 5-amino-2-methoxybenzoic acid, and oxalic acid groups. We have designated this amino acid by the three-letter abbreviation "Hao" to reflect these three components. Scheme 2 illustrates its structure and shows its relationship to a tripeptide.

Scheme 1



Scheme 2



This paper reports our studies of the amino acid Hao: the preparation of its *N*-protected derivatives, the use of these derivatives in peptide synthesis, and the remarkable propensity of a Hao-containing peptide to form a β-sheetlike dimer.

Results

Hao is readily prepared as its 2,7-di-*tert*-butylfluorenylmethyloxycarbonyl protected derivative (**8**) by the condensation of suitably protected derivatives of hydrazine, 5-amino-2-methoxybenzoic acid, and oxalic acid, as shown in Scheme 3. The 2,7-di-*tert*-butylfluorenylmethyloxycarbonyl (Fmoc*) group is used in place of the popular fluorenylmethyloxycarbonyl (Fmoc) group to improve the solubility of Hao and its precursors in organic solvents.[18] Reaction of 2,7-di-*tert*-butylfluorenylmethyloxycarbonyl chloride (Fmoc*-Cl) with anhydrous hydrazine affords Fmoc*-hydrazine (**3**). Coupling with 2-methoxy-5-nitrobenzoyl chloride (**4**)[16c] gives hydrazide **5**. Reduction of the nitro group generates amine **6**. Condensation with ethyl oxalyl chloride gives amide **7**. Hydrolysis of the ethyl ester group with NaOH, followed by passage of the reaction mixture through acidic ion exchage resin, yields Fmoc*-Hao (**8**). When this procedure was performed on a multigram scale, a 75% yield for the conversion of Fmoc*-Cl to Fmoc*-Hao was obtained. The Boc-protected derivative of Hao (Boc-Hao) was prepared in a similar fashion from Boc-hydrazine.

(7) (a) Roberts, J. C.; Pallai, P. V.; Rebek, J., Jr. *Tetrahedron Lett.* **1995**, *36*, 691−694. (b) Boumendjel, A.; Roberts, J. C.; Hu, E.; Pallai, P. V.; Rebek, J., Jr. *J. Org. Chem.* **1996**, *61*, 4434−4438.

(8) (a) Schrader, T.; Kirsten, C. *Chem. Commun.* **1996**, 2089−2090. (b) Kirsten, C. N.; Schrader, T. H. *J. Am. Chem. Soc.* **1997**, *119*, 12061−12068.

(9) (a) Smith, A. B., III; Guzman, M. C.; Sprengeler, P. A.; Keenan, T. P., Holcomb, R. C.; Wood, J. L.; Carroll, P. J.; Hirschmann, R. *J. Am. Chem. Soc.* **1994**, *116*, 9947−9962. (b) Smith, A. B., III; Hirschmann, R.; Pasternak, A.; Guzman, M. C.; Yokoyama, A.; Sprengeler, P. A.; Darke, P. L.; Emini, E. A.; Schleif, W. A. *J. Am. Chem. Soc.* **1995**, *117*, 11113−11123. (c) Smith, A. B., III; Benowitz, A. B.; Sprengeler, P. A.; Barbosa, J.; Guzman, M. C.; Hirschmann, R.; Schweiger, E. J.; Bolin, D. R.; Nagy, Z.; Campbell, R. M.; Cox, D. C.; Olson, G. L. *J. Am. Chem. Soc.* **1999**, *121*, 9286−9298.

(10) (a) Abbenante, G.; March, D. R.; Bergman, D. A.; Hunt, P. A.; Garnham, B.; Dancer, R. J.; Martin, J. L.; Fairlie, D. P. *J. Am. Chem. Soc.* **1995**, *117*, 10220−10226. (b) March, D. R.; Abbenante, G.; Bergman, D. A.; Brinkworth, R. I.; Wickramasinghe, W.; Begun, J.; Martin, J. L.; Fairlie, D. P. *J. Am. Chem. Soc.* **1996**, *118*, 3375−3379. (c) Reid, R. C.; March, D. R.; Dooley, M. J.; Bergman, D. A.; Abbenante, G.; Fairlie, D. P. *J. Am. Chem. Soc.* **1996**, *118*, 8511−8517.

(11) Janetka, J. W.; Raman, P.; Satyshur, K.; Flentke, G. R.; Rich, D. H. *J. Am. Chem. Soc.* **1997**, *119*, 441−442.

(12) Zutshi, R.; Franciskovich, J.; Shultz, M.; Schweitzer, B.; Bishop, P.; Wilson, M.; Chmielewski, J. *J. Am. Chem. Soc.* **1997**, *119*, 4841−4845.

(13) Nowick, J. S.; Smith, E. M.; Pairish, M. *Chem. Soc. Rev.* **1996**, *25*, 401−415.

(14) (a) Kemp, D. S.; Bowen, B. R. *Tetrahedron Lett*. **1988**, *29*, 5077−5080. (b) Kemp, D. S.; Bowen, B. R. *Tetrahedron Lett*. **1988**, *29*, 5081−5082. (c) Kemp, D. S.; Bowen, B. R.; Muendel, C. C. *J. Org. Chem.* **1990**, *55*, 4650−4657.

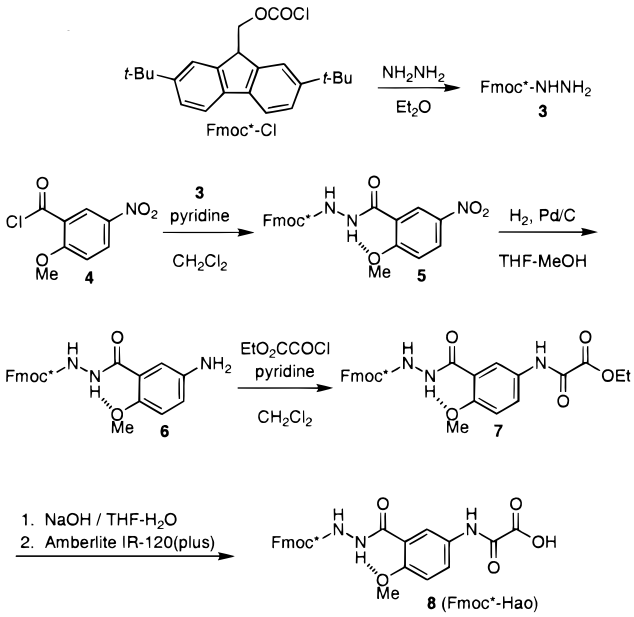
(15) Nowick, J. S. *Acc. Chem. Res.* **1999**, *32*, 287−296.

(16) (a) Nowick, J. S.; Smith, E. M.; Noronha, G. *J. Org. Chem.* **1995**, *60*, 7386-7387. (b) Nowick, J. S.; Holmes, D. L.; Mackin, G.; Noronha, G.; Shaka, A. J.; Smith, E. M. *J. Am. Chem. Soc.* **1996**, *118*, 2764−2765. (c) Nowick, J. S.; Pairish, M.; Lee, I. Q.; Holmes, D. L.; Ziller, J. W. *J. Am. Chem. Soc.* **1997**, *119*, 5413−5424. (d) Holmes, D. L.; Smith, E. M.; Nowick, J. S. *J. Am. Chem. Soc*. **1997**, *119*, 7665−7669. (e) Smith, E. M.; Holmes, D. L.; Shaka, A. J.; Nowick, J. S. *J. Org. Chem.* **1997**, *62*, 7906−7907. (f) Tsai, J. H.; Waldman, A. S.; Nowick, J. S. *Bioorg. Med. Chem.* **1999**, *7*, 29−38. (g) Junquera, E.; Nowick, J. S. *J. Org. Chem.* **1999**, *64*, 2527−2531.

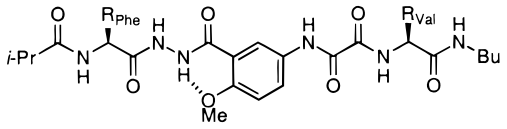
(17) Nowick, J. S.; Tsai, J. H.; Bui, Q.-C. D.; Maitra, S. *J. Am. Chem. Soc*. **1999**, *121*, 8409−8410.

(18) Stigers, K. D.; Koutroulis, M. R.; Chung, D. M.; Nowick, J. S. *J. Org. Chem.* **2000**, *65*, 3858−3860.

Scheme 3



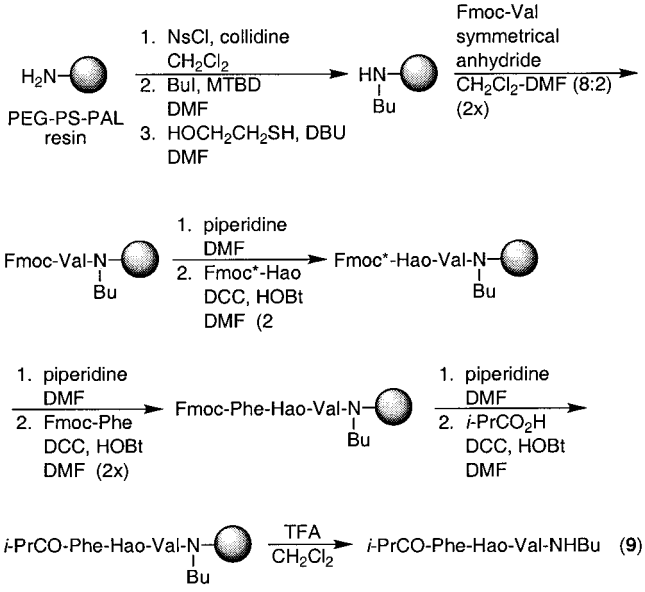
To evaluate the synthetic and structural properties of Hao, we used these Hao derivatives to prepare the tripeptide *i*-PrCO-Phe-Hao-Val-NHBu (**9**) by several different methods. We chose this compound as a simple, nonfunctionalized peptide derivative that would be suitable for ^{1}H NMR studies in chloroform solution. Initially, we had prepared the corresponding methylamide, *i*-PrCO-Phe-Hao-Val-NHMe. However, the limited (low millimolar) solubility of this compound made its study by NMR inconvenient. For this reason, we performed all subsequent studies on the more soluble butylamide.



9 (*i*-PrCO-Phe-Hao-Val-NHBu)

We first prepared tripeptide **9** manually on poly(ethylene glycol)−polystyrene (PEG-PS) resin with a tris(alkoxy)benzylamide (PAL) linker[19] using a modified version of the backbone amide linker (BAL) strategy described by Barany, Albericio, and co-workers.[20] In our modification, we introduced the butyl group by converting the PAL amino group to its *o*-nitrobenzenesulfonamide by treatment with *o*-nitrobenzenesulfonyl chloride (NsCl), alkylation with *n*-butyl iodide and 1,3,4,6,7,8-hexahydro-1-methyl-2*H*-pyrimido[1,2-*a*]pyrimidine (MTBD), and removal of the nitrobenzenesulfonamide group with mercaptoethanol and DBU.[21,22] As described by Barany, Albericio, and co-workers, coupling of Fmoc-valine to the resulting sterically hindered (Bu)PAL-PEG-PS proved difficult using

Scheme 4



standard coupling reagents, but proceeded smoothly using the symmetrical anhydride in CH_2Cl_2−DMF (8:2).[20] The Hao and Phe residues were introduced by double coupling with DCC and HOBt; the isobutryic acid residue was introduced by single coupling under similar conditions.[23,24] Cleavage from the resin with TFA then afforded tripeptide **9**. Scheme 4 illustrates this synthesis.

When "indole resin" recently became commercially available, we tried using it to prepare tripeptide **9**.[25] This resin bears an indole-3-carboxaldehyde linker, which allows the butylamine portion of **9** to be introduced by reductive amination. Subsequent introduction of the amino acid residues with DCC and HOBt proceeded smoothly. The product produced by this route proved slightly more pure than that generated on the PEG-PS resin with the PAL linker. For this reason, and because of the absence of the need of the symmetrical anhydride, we consider this linker superior and have adopted it for subsequent solid-phase syntheses of related *N*-alkylamides.

To evaluate the behavior of Hao in solution-phase syntheses, we synthesized peptide **9** in solution by sequential coupling of Val-NHBu, Boc-Hao, Boc-Phe, and isobutyric acid using 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and HOBt in a mixture of THF and DMF. This synthesis also proceeded smoothly, although the limited solubility of the Hao-containing intermediates necessitated using DMF as a cosolvent. Collectively, these studies show that Fmoc*-Hao and Boc-Hao behave like regular amino acids in both solid-phase and solution-phase peptide syntheses with carbodiimide coupling agents.[26]

To evaluate the effect of Hao upon peptide structure, we studied tripeptide **9** by ^{1}H NMR spectroscopy. ^{1}H NMR chemical shift, NOE, and dilution titration studies indicate that Hao derivative **9** forms a remarkable β-sheetlike hydrogen-

(19) (a) Albericio, F.; Barany, G. *Int. J. Pept. Protein Res.* **1987**, *30*, 206−216. (b) Albericio, F.; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hudson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730−3743.

(20) (a) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vágner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441−5452. (b) Alsina, J.; Jensen, K. J.; Albericio, F.; Barany, G. *Chem. Eur. J.* **1999**, *5*, 2787−2795.

(21) (a) Miller, S. C.; Scanlan, T. S. *J. Am. Chem. Soc.* **1997**, *119*, 2301−2302. (b) Miller, S. C.; Scanlan, T. S. *J. Am. Chem. Soc.* **1998**, *120*, 2690−2691.

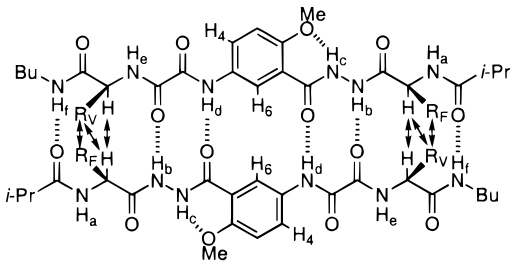
(22) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373−6374.

(23) Interestingly, we obtained better coupling of Fmoc*-Hao using DCC and HOBt than using HATU or HATU and HOAt, which are often better peptide coupling agents. For descriptions of HATU and HOAt, see: Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.

(24) Our experiences suggest that the coupling of Hao is slightly less efficient than that of ordinary amino acids. Double coupling was performed in each step in the synthesis to ensure complete reaction, although it was not clear that it was required.

(25) Estep, K. G.; Neipp, C. E.; Stephens Stramiello, L. M.; Adam, M. D.; Allen, M. P.; Robinson, S.; Roskamp, E. J. *J. Org. Chem.* **1998**, *63*, 5300−5301.

Scheme 5



bonded dimer in $CDCl_3$ solution. Scheme 5 illustrates the structure of this dimer.

In this structure, NH protons H_b, H_c, H_d, and H_f are hydrogen-bonded, while NH protons H_a and H_e are not. In the ^1H NMR spectrum in $CDCl_3$ solution, hydrogen-bonded NH protons typically appear about 2 ppm downfield of non-hydrogen-bonded NH protons. Peptide amides generally appear at ~6 ppm when not hydrogen-bonded and at ~8 ppm when hydrogen-bonded.[13] In Hao derivative **9**, the chemical shift of the (non-hydrogen-bonded) phenyalanine NH_a group is 6.35 ppm in 7 mM $CDCl_3$ solution at 295 K, while that of the (hydrogen-bonded) valine butylamide NH_f group is 7.93 ppm. The three NH protons of Hao, H_b, H_c, and H_d appear at 11.74, 11.18, and 10.71 ppm, respectively. These values are comparable to those of the corresponding hydrogen-bonded protons in artificial β-sheets **1a** and **b** (11.3, 11.2, and 10.7 ppm, respectively).[17] The remaining NH proton (H_e) appears at 8.41 ppm. This proton belongs to an oxalamide system and appears significantly downfield of that of the NH proton of *N*,*N*′-dibutyloxalamide in dilute $CDCl_3$ solution (7.44 ppm, 7 mM), conditions at which it is not hydrogen-bonded. The downfield shifting of this proton relative to the dibutyloxalamide control appears too small to arise from a typical hydrogen bond, and may instead result from magnetic anisotropy associated with the valine carbonyl group and Hao aromatic ring.

The chemical shifts of the amino acid α-protons of **9** are also consistent with the dimeric β-sheetlike structure shown in Scheme 5. Protein α-protons generally resonate downfield of those in random coils by several tenths of a ppm in β-sheets.[27] In **9**, the Phe and Val α-protons appear at 5.38 and 4.62 ppm respectively, substantially downfield of the corresponding random coil values (4.66 and 3.95 ppm). Also consistent with the dimeric structure, the chemical shift of the Phe α-proton in **9** is comparable to the limiting chemical shift of the Phe α-proton in the dimer of artificial β-sheet **1a** (5.29 ppm).[17]

Interstrand NOES are a hallmark of β-sheets.[28] ^1H NMR transverse-ROESY (Tr-ROESY)[29] studies in $CDCl_3$ solution (7 mM, 30 °C) show that Hao derivative **9** exhibits strong NOEs between the Phe and Val α-protons and weaker NOEs between the Val γ (methyl) protons and the Phe α, β, and δ protons. Scheme 5 illustrates these NOEs with arrows. These long-range NOEs cannot easily be explained by intramolecular contacts, but are wholly consistent with the dimeric structure.

Intrastrand NOE data also provide evidence that **9** adopts a β-strandlike conformation. Notably, the aromatic amino proton (H_d) exhibits a strong NOE with the aromatic H_6 proton but exhibits no NOE with the aromatic H_4 proton. Similarly, the Val α-proton exhibits a stronger interresidue NOE with butylamide proton H_f and a weaker NOE with the Val NH proton (H_e). When a 300 ms mixing time is used in the Tr-ROESY experiment, the Phe α-proton exhibits weak inter- and intraresidue NOEs with both the Hao NH proton H_b and the Phe NH proton (H_a). The weakness of the interresidue NOE appears to result from an unusually short transverse relaxation time (and associated $T_{1\rho}$) for the Hao NH proton H_b. The short relaxation time of this proton, evidenced by the broadness of its peak in the ^1H NMR spectrum, should result in loss of phase coherence during the mixing period of the Tr-ROESY experiment and generate an anomalously weak NOE. Consistent with this explanation, the Phe α-proton gives a relatively strong interresidue NOE with the Hao NH proton H_b and little or no intraresidue NOE with the Phe NH proton (H_a) when a shorter (100 ms) mixing time is used. Coupling constant data provide further evidence for a β-strandlike conformation, with $^3J_{HN\alpha}$ values of 8.4 Hz (Phe) and 9.6 Hz (Val).[13,30]

Peptide derivative **9** is too strongly dimerized in pure $CDCl_3$ to allow its dimerization constant to be accurately determined by ^1H NMR dilution titration studies. The NH groups H_a, H_b, and H_c of **9** exhibit very little concentration dependence in chemical shift (0.05−0.07 ppm from 0.16 to 2.6 mM) in $CDCl_3$ solution and show saturation at higher concentrations. These data indicate that **9** is virtually completely dimerized at NMR accessible concentrations. Analysis of this very limited titration data set reveals a dimerization constant of $\sim 10^6$ M^{-1}. This value is dramatically larger than that of the tripeptide *i*-PrCO-Phe-Leu-Val-NHBu, which was prepared as a control and found to have a dimerization constant of 100−200 M^{-1} by fitting dimerization isotherms to the NH and α-proton chemical shifts at varying concentrations. Several pentapeptides were also prepared as controls but were found to be too insoluble for ^1H NMR titration studies.

Addition of the competitive solvent CD_3OD to the $CDCl_3$ weakens the dimerization of **9**, allowing a dimerization constant to be determined by ^1H NMR dilution titration studies. Thus, dilution titration studies, in which the chemical shifts of the α-protons were monitored as a function of concentration and a dimerization isotherm was fitted to the shift data, reveal a dimerization constant (K_{dim}) of 900 M^{-1} in 10% CD_3OD−$CDCl_3$.[31−33] Figure 2 illustrates these data and the fitted isotherms. In this solvent system, the control tripeptide *i*-PrCO-

(26) Hao can also be constructed during the convergent synthesis of larger molecules that contain it. Thus, we have also prepared **9** convergently, by forming its oxalic acid amide bond using EDC (*i*-PrCO-Phe-NHNH-$COArNH_2$ + HO_2CCO-Val-NHBu → **9**). In addition, we have found that simple esters of Hao and its derivatives are exceptionally reactive, allowing their facile direct coupling with simple amines, even in dilute solution (e.g., *i*-PrCO-Phe-Hao-OEt + RNH_2 → *i*-PrCO-Phe-Hao-NHR). This exceptional reactivity of Hao ester derivatives also allows their facile base−promoted hydrolysis and transesterification.

(27) (a) Wishart, D. S.; Sykes, B. D.; Richards, F. M. *J. Mol. Biol.* **1991**, *222*, 311−333. (b) Wishart, D. S.; Sykes, B. D.; Richards, F. M. *Biochemistry* **1992**, *31*, 1647−1651.

(28) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986; pp 125−129.

(29) (a) Hwang, T.-L.; Shaka, A. J. *J. Am. Chem. Soc.* **1992**, *114*, 3157−3159. (b) Hwang, T.-L.; Shaka, A. J. *J. Magn. Reson., Ser. B* **1993**, *102*, 155−165.

(30) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986; pp 166−168.

(31) The titration data were analyzed by nonlinear least-squares fitting the following equation to the data: $\delta_{obs} = \delta_{free}((1 + 8K_{dim}[\mathbf{9}]_{tot})^{1/2} - 1)/4K_{dim}[\mathbf{9}]_{tot} + \delta_{bound}(4K_{dim}[\mathbf{9}]_{tot} - (1 + 8K_{dim}[\mathbf{9}]_{tot})^{1/2} + 1)/4K_{dim}[\mathbf{9}]_{tot}$, where δ_{obs} = observed chemical shift, δ_{free} = chemical shift of **9**, δ_{bound} = chemical shift of dimer, $[\mathbf{9}]_{tot}$ = total concentration of **9** in solution, and K_{dim} = the equilibrium constant for formation of the dimer complex. The quantities δ_{free}, δ_{bound}, and K_{dim} were allowed to vary during the fitting procedure, the δ_{obs} was measured during the titration, and the quantity $[\mathbf{9}]_{tot}$ was calculated from the volumes and concentrations of the solutions used in the titration.

(32) The value of K_{dim} (900 M^{-1}) was estimated to be accurate to within ±100 on the basis of multiple independent titrations. For the Val α-proton, δ_{free} and δ_{bound} were calculated to be 4.11 and 4.58 ppm, respectively; for the Phe α-proton, δ_{free} and δ_{bound} were calculated to be 4.83 and 5.26 ppm, respectively.

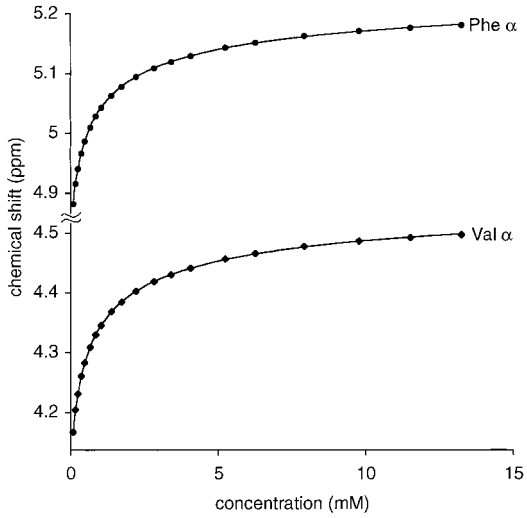
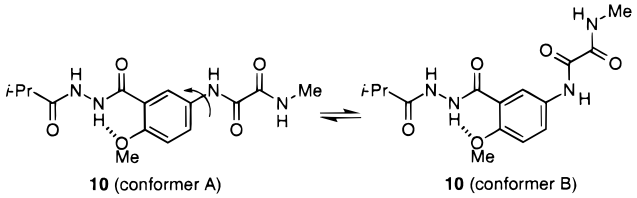


Figure 2. ^{1}H NMR chemical shift of α-protons of **9** as a function of concentration in $CDCl_3$ solution at 295 K. The curves are dimerization isotherms that best fit the data points.

Scheme 6



Phe-Leu-Val-NHBu self-associates too weakly to allow accurate determination of its dimerization constant by ^{1}H NMR titration. Analysis of the limited data sets available from ^{1}H NMR dilution titration revealed a dimerization constant of $\sim 5\ M^{-1}$.

Attempts to crystallize **9** to determine its solid-state structure by X-ray crystallography proved unsuccessful. A smaller homologue *i*-PrCO-Hao-NHMe (**10**) did crystallize but did not form β-sheetlike hydrogen-bonded dimers in the solid state. Instead of adopting a β-strandlike conformation that could form β-sheetlike dimers (Scheme 6, conformer A), this compound adopted a conformation in which rotation about the Ar−NH bond of the Hao unit occurred (Scheme 6, conformer B). These results provide a reminder that although Hao is more conformationally constrained than a peptide, it can adopt a conformation that is not β-strandlike as well as one that is.[34]

Discussion

Within the past couple of years, considerable interest in developing systems that dimerize strongly has emerged. Leading examples have been developed in the laboratories of Meijer,[35] Zimmerman,[36] and Gong.[37] Peptide derivative **9** is similar to these systems in that it dimerizes with comparable strength. One difference is that the dimerization interface of peptide derivative **9** is longer and has six intermolecular hydrogen bonds, while the examples described above have four. More significantly, **9** differs from these other systems in that its key structural unit is an unnatural amino acid that can be combined with natural amino acids to give hybrid peptides that are complementary to protein β-sheets.

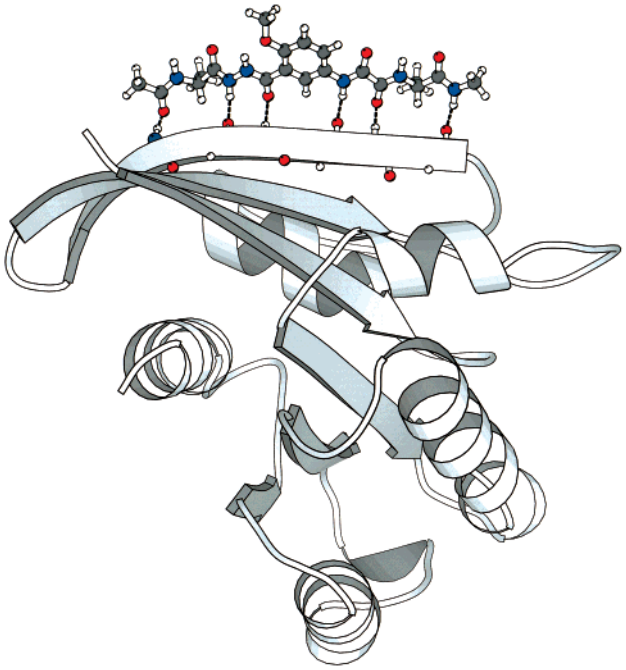


Figure 3. Molecular model of Ac-Ala-Hao-Ala-NHMe docked to the β-sheet edge of the Ras analogue, Rap1A. Modeling was performed using MacroModel V6.5 with the AMBER* force field and the figure was rendered using Molscript.[5]

This unnatural amino acid, Hao, imparts a β-sheetlike conformation to peptides that contain it and facilitates their dimerization through β-sheet interactions. In hybrid peptide **9**, Hao provides a β-strandlike edge with an alternating array of hydrogen-bond donors and acceptors that is preorganized to dimerize. Unlike peptides composed solely of natural amino acids, Hao-containing peptides do not readily form higher oligomers, because the aromatic ring of Hao blocks the other edge of the peptide strand.

Artificial β-sheets **1** can also be viewed as hybrid peptides, consisting of the tripeptide mimic Hao, the turn forming dipeptide replacement, $-N(Ph)CH_2CH_2N(CH_2CH_2CN)CO-$, and a tripeptide region; written as a peptide, the structure of **1a** is *i*-PrCO-Hao-N(Ph)CH_2CH_2N(CH_2CH_2CN)CO-Phe-Ile-Leu-NHMe. In artificial β-sheets **1**, Hao serves as a template that organizes the tripeptide region into a β-sheet that can dimerize. Thus Hao imparts both structure (conformation and folding) and function (dimerization) to peptides that contain it.

Hybrid peptides containing Hao display hydrogen-bonding surfaces that are complementary to the hydrogen-bonding edges of protein β-sheets. Figure 3 illustrates this complementarity through a model of Hao-containing peptide Ac-Ala-Hao-Ala-NHMe docked to the β-sheet edge of the Ras analogue, Rap1A. The edge of this simple tripeptide provides an alternating pattern of hydrogen-bond donors and acceptors that matches that of the protein. An intriguing application of Hao would be to incorporate it into peptides that bind to the edges of protein β-sheets. Such peptides offer the appealing promise of blocking β-sheet interactions between proteins. To achieve this promise, we will have to learn how to make Hao-containing peptides

(33) Interestingly, peptide derivative **9** shows a strong propensity to form dimers in the electrospray mass spectrum, and the relative intensities of the dimer and monomer peaks correlate with the concentration of **9** introduced into the spectrometer.

(34) Although these X-ray crystallographic studies show that **10** adopts this alternative conformation in the solid state, the ^{1}H NMR Tr-ROESY studies establish that **9** does not adopt this alternative conformation in its dimer in $CDCl_3$ solution.

(35) Beijer, F. H.; Kooijman, H.; Spek, A. L.; Sijbesma, R. P.; Meijer, E. W. *Angew. Chem., Int. Ed.* **1998**, *37*, 75−78. (b) Beijer, F. H.; Sijbesma, R. P.; Kooijman, H.; Spek, A. L.; Meijer, E. W. *J. Am. Chem. Soc.* **1998**, *120*, 6761−6769.

(36) Corbin, P. S.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1998**, *120*, 9710−9711.

that selectively recognize other molecules in preference to dimerizing and we will have to achieve this recognition in aqueous solution. Studies directed toward these goals are underway in our laboratories and will be reported in due course.

Conclusions

In summary, the unnatural amino acid Hao is a tripeptide β-strand mimic that reproduces the hydrogen-bonding pattern of one edge of a peptide β-strand. Fmoc*-Hao and Boc-Hao behave like typical Fmoc- and Boc-protected amino acids and can be incorporated into peptides by standard peptide synthesis techniques. The resulting peptide−peptidomimetic hybrid compounds form β-sheetlike dimers. We anticipate that hybrid peptides containing Hao may serve as antagonists to block β-sheet interactions between proteins. We will pursue this idea in future studies.

Experimental Section

General. Commercial grade reagents and solvents were used without further purification. CH_2Cl_2 and THF were dried prior to use by passage through anhydrous Al_2O_3 as described by Grubbs and co-workers;[38] DMF was dried in an analogous fashion by percolation through 3 Å molecular sieves.[39] PS-PEG-PAL-Fmoc resin was obtained from PE Biosystems. High-resolution mass spectra were obtained by liquid secondary-ion ionization (LSI) of samples in a *m*-nitrobenzyl alcohol matrix bombarded with Cs^+ ions at 25 kV (instrumental variation $\sigma =$ 2 mmu). All solution-phase reactions were performed with magnetic stirring; moisture-sensitive solution-phase reactions were carried out in flame- or oven-dried glassware under nitrogen. Solid-phase reactions were performed with mechanical shaking and were, where appropriate, monitored by qualitative ninhydrin tests.[40] Reaction mixtures and product solutions were concentrated by rotary evaporation; where appropriate, the residue was further dried using a vacuum pump.

Fmoc*-hydrazine (3). A chilled (0 °C) solution of Fmoc*-Cl[18] (18.72 g, 50.47 mmol) in ether (150 mL) was added to a stirred, ice-cooled solution of anhydrous hydrazine (6.40 mL, 202 mmol) in ether (100 mL) over 2 min.[41] The ice-bath was removed, and the solution was stirred for 3 h. The reaction mixture was transferred to a separatory funnel with ether (50 mL) and washed with water (3 × 150 mL) followed by saturated aqueous NaCl (150 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to afford 18.40 g (100%) of Fmoc*-hydrazine (**3**) as a white foamy solid: mp 152−154 °C; IR ($CHCl_3$) 3450, 3352, 1726, 1632 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, $J = 8.0$ Hz, 2 H), 7.57 (br s, 2 H), 7.41 (dd, $J = 8.0$, 1.6 Hz, 2 H), 6.11 (br s, 1 H), 4.43 (d, $J = 7.1$ Hz, 2 H), 4.17 (t, $J = 6.8$ Hz, 1 H), 3.78 (br s, 2 H), 1.37 (s, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.7, 149.9, 143.7, 138.7, 124.8, 121.8, 119.2, 67.8, 47.2, 34.9, 31.6; HRMS (LSIMS) *m*/*z* for $C_{23}H_{30}N_2O_2$ $[M]^+$ calcd 366.2307, found 366.2293. Anal. Calcd for $C_{23}H_{30}N_2O_2$: C, 75.38; H, 8.25; N, 7.64. Found: C, 75.53; H, 7.96; N, 7.59.

Hydrazide 5. 2-Methoxy-5-nitrobenzoic acid[16c] (4.33 g, 22.0 mmol) was stirred with oxalyl chloride (5.80 mL, 66.0 mmol) and DMF (10 μL) in THF (50 mL) for 1 h. The solvent was removed, and a solution of the resulting acid chloride in CH_2Cl_2 (50 mL) was added over ∼2 min to a stirred, ice-cold solution of Fmoc*-hydrazine (**3**) and pyridine (1.87 mL, 23.1 mmol) in CH_2Cl_2 (80 mL). The ice-bath was removed and the resulting solution was stirred for 30 min. The reaction mixture was washed with H_2O (150 mL), saturated aqueous $NaHCO_3$ (150 mL), and saturated aqueous NaCl (150 mL), dried over $MgSO_4$, filtered, and concentrated to yield 11.70 g of hydrazide **5** (99%) as a white fluffy solid. An analytical sample was purified by column chromatography on silica gel: mp 126−127 °C; IR ($CHCl_3$) 3410, 1749, 1682, 1616 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.42 (br s, 1 H), 9.08 (br s, 1 H), 8.38 (dd, $J = 9.1$, 3.0 Hz, 1 H), 7.60 (br s, 4 H), 7.41 (d, $J = 6.7$ Hz, 2 H), 7.22 (br s, 1 H), 7.12 (d, $J = 9.2$ Hz, 1 H), 4.51 (d, $J = 7.0$ Hz, 2 H), 4.22 (t, $J = 6.8$ Hz, 1 H), 4.11 (br s, 3 H), 1.37 (s, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.2, 161.6, 156.0, 150.0, 143.5, 142.1, 138.7, 128.9, 128.8, 124.9, 121.9, 120.2, 119.2, 112.0, 68.6, 57.3, 47.0, 34.9, 31.6; HRMS (LSIMS) *m*/*z* for $C_{31}H_{35}N_3O_6$ $[M]^+$ calcd 545.2526, found 545.2530. Anal. Calcd for $C_{31}H_{35}N_3O_6$: C, 68.24; H, 6.47; N, 7.70. Found: C, 68.13; H, 6.47; N, 7.75.

Amine 6. A 500-mL, three-necked, round-bottomed flask equipped with a stopper, a septum, a magnetic stirring bar, and a three-way stopcock connected to a vacuum line and to a balloon filled with hydrogen was charged with hydrazide **5** (11.70 g, 21.44 mmol), 10% Pd/C (1.20 g), CH_3OH (100 mL) and THF (200 mL). The flask was evacuated and filled with hydrogen (3×), and the reaction mixture was allowed to stir under hydrogen. After 2 h, the flask was evacuated and opened to air, the suspension was filtered through Celite, and the Celite bed was rinsed thoroughly with ethyl acetate (150 mL). The filtrate was evaporated to dryness to obtain 11.01 g of an off-white solid. Purification by column chromatography (gradient elution with EtOAc: hexanes, 1:20−3:1, on a 7.5 cm d × 19 cm h column of silica gel) yielded 8.60 g (76% from Fmoc*-Cl) of amine **6** as a white fluffy solid: mp 108−121 °C; IR ($CHCl_3$) 3392, 1736, 1668, 1624 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.72 (br s, 1 H), 7.64−7.62 (m, 4 H), 7.55 (dd, $J = 2.5$, 0.8 Hz, 1 H), 7.41 (d, $J = 7.4$ Hz, 2 H), 7.18 (br s, 1 H), 6.85−6.81 (m, 2 H), 4.48 (d, $J = 7.3$ Hz, 2 H), 4.23 (t, $J = 7.3$ Hz, 1 H), 3.92 (s, 3 H), 3.56 (br s, 2 H), 1.36 (s, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.5, 156.2, 150.8, 149.9, 143.7, 140.7, 138.7, 124.8, 122.0, 120.2, 119.6, 119.2, 118.4, 113.0, 68.5, 56.6, 47.1, 34.9, 31.6; HRMS (LSIMS) *m*/*z* for $C_{31}H_{37}N_3O_4$ $[M]^+$ calcd 515.2784, found 515.2799. Anal. Calcd for $C_{31}H_{37}N_3O_4$: C, 72.21; H, 7.23; N, 8.15. Found: C, 72.04; H, 7.30; N, 8.15.

Amide 7. To an ice-cooled solution of amine **6** (8.60 g, 16.7 mmol) and pyridine (1.60 mL, 19.8 mmol) in CH_2Cl_2 (150 mL), was added ethyl oxalyl chloride (2.10 mL, 18.8 mmol) in drops over 2 min. After 15 min, the solution was transferred to a separatory funnel with CH_2Cl_2 (50 mL), washed with H_2O (150 mL) and saturated aqueous NaCl solution (150 mL), dried over $MgSO_4$, filtered, and concentrated to yield 10.17 g (99%) of amide **7** as a white solid: mp 222−225 °C; IR ($CHCl_3$) 3390, 1732, 1710, 1672 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.74 (br s, 1 H), 9.43 (br s, 1 H), 8.37 (d, $J = 8.8$ Hz, 1 H), 8.26 (d, $J = 2.8$ Hz, 1 H), 7.63−7.60 (m, 5 H), 7.41−7.39 (m, 2 H), 7.05 (d, $J = 9.2$ Hz, 1 H), 4.48 (d, $J = 7.2$ Hz, 2 H), 4.41 (q, $J = 7.2$ Hz, 2 H), 4.22 (t, $J = 7.0$ Hz, 1 H), 4.01 (s, 3 H), 1.40 (t, $J = 7.4$ Hz, 3 H), 1.35 (s, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.5, 161.1, 156.0, 154.7, 154.2, 149.9, 143.6, 138.6, 131.0, 125.5, 124.8, 124.2, 121.9, 119.4, 119.2, 112.2, 68.4, 63.7, 56.5, 47.0, 34.8, 31.7, 13.9; HRMS (LSIMS) *m*/*z* for $C_{35}H_{41}N_3O_7$ $[M]^+$ calcd 615.2944, found 615.2953. Anal. Calcd for $C_{35}H_{41}N_3O_7$: C, 68.27; H, 6.71; N, 6.82. Found: C, 68.53; H, 6.57; N, 6.62.

Fmoc*-Hao (8). To a solution of **7** (10.17 g, 16.52 mmol) in THF: H_2O (300 mL, 4:1) was added 1.00 M NaOH solution (16.55 mL, 16.55 mmol) in a single portion. After 30 min, the solution was passed through a column of Amberlite IR-120(plus) ion-exchange resin (4 cm d × 15 cm h, 100 mL, 1.9 mmol/mL) and concentrated to yield 9.62 g (99%) of Fmoc*-Hao (**8**) as a light tan solid: mp 168−183 °C; IR (KBr) 3700−2400 (br), 3377, 1738, 1693 cm^{-1}; ^1H NMR (500 MHz, CD_3SOCD_3) δ 14.10 (br s, 1 H), 10.81 (s, 1 H), 9.85 (s, 1 H), 9.60 (s, 1 H), 8.17 (d, $J = 2.5$ Hz, 1 H), 7.88 (dd, $J = 9.0$, 2.6 Hz, 1 H), 7.77 (s, 2 H), 7.75 (d, $J = 8.1$ Hz, 2 H), 7.45 (d, $J = 8.0$ Hz, 2 H), 7.17 (d, $J = 9.1$ Hz, 1 H), 4.35 (d, $J = 7.3$ Hz, 2 H), 4.24 (t, $J = 7.2$ Hz, 1 H), 3.87 (s, 3 H), 1.37 (s, 18 H); ^{13}C NMR (125 MHz, CD_3SOCD_3) δ 164.9, 162.1, 156.6, 156.2, 153.7, 149.5, 143.8, 138.1, 130.8, 124.6, 124.6, 122.6, 122.1, 121.7, 119.3, 112.3, 66.8, 56.1, 46.6, 34.7, 31.4; HRMS (LSIMS) *m*/*z* for $C_{33}H_{37}N_3O_7$ $[M]^+$ calcd 587.2631, found 587.2631. Anal. Calcd for $C_{33}H_{37}N_3O_7$: C, 67.45; H, 6.35; N, 7.15. Found: C, 67.67; H, 6.23; N, 6.90.

(37) Gong, B.; Yan, Y.; Zeng, H.; Skrzypczak-Jankunn, E.; Kim, Y. W.; Zhu, J.; Ickes, H. *J. Am. Chem. Soc.* **1999**, *121*, 5607−5608.

(38) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518−1520.

(39) Burfield, D. R.; Smithers, R. H. *J. Org. Chem.* **1978**, *43*, 3966−3968.

(40) Stewart, J. M.; Young, J. D. *Solid-Phase Peptide Synthesis*; Pierce Chemical Company: Rockford, Illinois, 1984; pp 105−106.

(41) Addition of the Fmoc*-Cl to the NH_2NH_2 is essential; addition of NH_2NH_2 to the Fmoc*-Cl resulted in substantially diminished yields of Fmoc*-$NHNH_2$.

Boc-Hao. To an ice-cooled solution of pure 5-NH_2-2-MeO-C_6H_3-CONHNHBoc[16f] (3.91 g, 13.9 mmol) and Et_3N (2.30 mL, 16.7 mmol) in CH_2Cl_2 (75 mL) was added ethyl oxalyl chloride (1.70 mL, 15.3 mmol) in drops over 1 min. After 15 min, the solution was transferred to a separatory funnel with CH_2Cl_2 (125 mL), washed with H_2O (100 mL) and saturated aqueous NaCl (100 mL), dried over $MgSO_4$ and the solvent was removed to yield 5.25 g (99%) of 5-EtO_2CCONH-2-MeO-C_6H_3-CONHNHBoc as a white solid: mp 184−185 °C; IR ($CHCl_3$) 3388, 1714, 1674 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.62−9.78 (m, 2 H), 8.37 (dd, J = 9.0, 2.8 Hz, 1 H), 8.33 (d, J = 2.8 Hz, 1 H), 7.47 (br s, 1 H), 7.02 (d, J = 9.1 Hz, 1 H), 4.44 (q, J = 7.2 Hz, 2 H), 4.00 (s, 3 H), 1.47 (s, 9 H), 1.44 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.2, 161.1, 155.1, 154.7, 154.2, 130.9, 125.3, 124.3, 119.5, 112.1, 81.7, 63.7, 56.5, 28.2, 14.0; HRMS (LSIMS) m/z for $C_{17}H_{23}N_3O_7$ $[M]^+$ calcd 381.1536, found 381.1533. Anal. Calcd for $C_{17}H_{23}N_3O_7$: C, 53.54; H, 6.08; N, 11.02. Found: C, 53.19; H, 5.76; N, 10.81.

To a solution of 5-EtO_2CCONH-2-MeO-C_6H_3-CONHNHBoc (2.45 g, 6.42 mmol) in THF:H_2O (120 mL, 4:1), was added 1.00 M NaOH solution (6.50 mL, 6.50 mmol) in a single portion. After 30 min, the solution was passed through a column of Amberlite IR-120(plus) ion-exchange resin (2.5 cm d × 10 cm h, 1.9 mmol/mL) and concentrated to yield 2.24 g (99%) of Boc-Hao as a white solid: mp 218−219 °C; IR (KBr) 3600−3400, 3336, 3273, 1720, 1691, 1649 cm^{-1}; ^{1}H NMR (500 MHz, CD_3SOCD_3) δ 10.75 (s, 1 H), 9.64 (s, 1 H), 8.93 (s, 1 H), 8.14 (d, J = 1.5 Hz, 1 H), 7.83 (dd, J = 8.8, 1.8 Hz, 1 H), 7.13 (d, J = 9.0 Hz, 1 H), 3.84 (s, 3 H), 1.41 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.8, 162.1, 156.6, 155.2, 153.8, 130.7, 124.6, 122.7, 121.7, 112.3, 79.1, 56.0, 28.1; HRMS (LSIMS) m/z for $C_{15}H_{19}N_3O_7$ $[M]^+$ calcd 353.1223, found 353.1230. Anal. Calcd for $C_{15}H_{19}N_3O_7$: C, 50.99; H, 5.42; N, 11.89. Found: C, 50.86; H, 5.39; N, 11.82.

Solid-Phase Synthesis of *i*-PrCO-Phe-Hao-Val-NHBu (9) on PEG-PS-PAL Resin. Alkylation of the Resin. PS-PEG-PAL-Fmoc resin (1.071 g, 0.175 mmol/g loading, 0.187 mmol) was shaken with three 10 mL-portions of 20% piperidine in DMF (10 min per treatment, draining between treatments), washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), CH_2Cl_2 and methanol (alternately, 3×, with 10 mL-portions of each solvent), CH_2Cl_2 (2 × 10 mL), ether (2 × 10 mL), and dried under a stream of dry nitrogen. The resin was then shaken with CH_2Cl_2 (10 mL), collidine (149 μL, 1.13 mmol), and 2-nitrobenzenesulfonyl chloride (125 mg, 0.565 mmol) for 3 h.[22] The solution was drained, and the resin was washed with CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), CH_2Cl_2 and methanol (alternately, 3×, with 10 mL-portions of each solvent), CH_2Cl_2 (2 × 10 mL), ether (2 × 10 mL) and dried under a stream of dry nitrogen. The resin was then shaken with DMF (10 mL); 1,3,4,6,7,8-hexahydro-1-methyl-2*H*-pyrimido[1,2-*a*]pyrimidine (MTBD, 108 μL, 0.752 mmol) and 1-iodobutane (215 μL, 1.89 mmol) for 8 h.[21] The solution was drained, and the resin was washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), CH_2Cl_2 and methanol (alternately, 3×, with 10 mL-portions of each solvent), CH_2Cl_2 (2 × 10 mL), ether (2 × 10 mL) and dried under a stream of dry nitrogen. The resin was then shaken with DMF (10 mL), DBU (140 μL, 0.939 mmol), and β-mercaptoethanol (130 μL, 1.86 mmol) for 3 h. The solution was drained, and this treatment was repeated to ensure complete deprotection. The resin was washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), CH_2Cl_2 and methanol (alternately, 3×, with 10 mL-portions of each solvent), CH_2Cl_2 (2 × 10 mL), ether (2 × 10 mL) and dried under a stream of dry nitrogen, to give PS-PEG-PAL(Bu).

Coupling of Val. DCC (0.393 g, 1.90 mmol) was added to a solution of 1.27 g of Fmoc-Val (3.74 mmol) in dichloromethane (47 mL). After 10 min, the resulting white suspension was filtered through a glass frit, the filtrate was concentrated, and the residue (Fmoc-Val anhydride) was suspended in 8:2 CH_2Cl_2:DMF (20 mL). The PS-PEG-PAL(Bu) resin was shaken with half (10 mL) of the suspension for 2 h, the solution was drained, and the resin was shaken with the other half (10 mL) of the suspension for 2 h.[20] The solution was drained, and the resin was washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), CH_2Cl_2 and methanol (alternately, 3×, with 10 mL-portions of each solvent), CH_2Cl_2 (2 × 10 mL), and ether (2 × 10 mL), and dried under a stream of dry nitrogen. The resin was shaken with three 10 mL-portions of 20% piperidine in DMF (10 min per treatment, draining between treatments), washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), CH_2Cl_2 and methanol (alternately, 3×, with 10 mL-portions of each solvent), CH_2Cl_2 (2 × 10 mL), ether (2 × 10 mL) and dried under a stream of dry nitrogen to give PS-PEG-PAL(Bu)-Val.

Coupling of Hao. The PS-PEG-PAL(Bu)-Val resin was shaken with DMF (10 mL), Fmoc*-Hao (**8**, 440 mg, 0.750 mmol), DCC (154 mg, 0.748 mmol), and HOBt·H_2O (101 mg, 0.66 mmol) for 2 h. The solution was then drained, and the coupling treatment of resin was repeated for an additional 2 h. The solution was drained, and the resin was washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), CH_2Cl_2 and methanol (alternately, 3×, with 10 mL-portions of each solvent), CH_2Cl_2 (2 × 10 mL), and ether (2 × 10 mL) and dried under a stream of dry nitrogen. The resin was shaken with three 10 mL-portions of 20% piperidine in DMF (10 min per treatment, draining between treatments), washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), CH_2Cl_2 and methanol (alternately, 3×, with 10 mL-portions of each solvent), CH_2Cl_2 (2 × 10 mL), ether (2 × 10 mL) and dried under a stream of dry nitrogen to give PS-PEG-PAL(Bu)-Val-Hao.

Coupling of Phe. The PS-PEG-PAL(Bu)-Val-Hao resin was shaken with DMF (10 mL), Fmoc-Phe (281 mg, 0.725 mmol), DCC (150 mg, 0.725 mmol), and HOBt·H_2O (98 mg, 0.64 mmol) for 2 h. The solution was then drained, and the coupling treatment of resin was repeated for an additional 3 h. The solution was drained, and the resin was washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), CH_2Cl_2 and methanol (alternately, 3×, with 10 mL-portions of each solvent), CH_2Cl_2 (2 × 10 mL), ether (2 × 10 mL) and dried under a stream of dry nitrogen. The resin was shaken with three 10 mL-portions of 20% piperidine in DMF (10 min per treatment, draining between treatments), washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), CH_2Cl_2 and methanol (alternately, 3×, with 10 mL-portions of each solvent), CH_2Cl_2 (2 × 10 mL), and ether (2 × 10 mL), and dried under a stream of dry nitrogen to give PS-PEG-PAL(Bu)-Val-Hao-Phe.

Coupling of Isobutyric Acid. The resin was shaken with DMF (10 mL), isobutyric acid (67 μL, 0.72 mmol), DCC (150 mg, 0.73 mmol), and HOBt·H_2O (100 mg, 0.65 mmol) for 1.5 h. The solution was drained, and the resin was washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), CH_2Cl_2 and methanol (alternately, 3×, with 10 mL-portions of each solvent), CH_2Cl_2 (2 × 10 mL), and ether (2 × 10 mL), and dried under a stream of dry nitrogen to give PS-PEG-PAL(Bu)-Val-Hao-Phe-CO-*i*-Pr.

Cleavage and Purification. The PS-PEG-PAL(Bu)-Val-Hao-Phe-CO-*i*-Pr resin (0.969 g) was allowed to stand with 10% TFA in CH_2Cl_2 (47 mL) for 1 h without agitation. The solution was filtered and concentrated, and the resultant oil was dissolved in CH_2Cl_2 (100 mL) and washed with saturated aqueous K_2CO_3 (100 mL). The organic layer was removed, dried over $MgSO_4$, filtered, and concentrated. The resultant solid (71 mg) was dissolved in hot MeOH (3 mL) and allowed to precipitate. The precipitate was collected in two crops by filtration and dried under vacuum to give 46 mg (39% yield, 46% corrected for resin losses) of *i*-PrCO-Phe-Hao-Val-NHBu (**9**) as a white solid: mp 152 °C dec; IR (KBr) 3442, 1641; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.73 (br s, 1 H), 11.18 (d, J = 7.8 Hz, 1 H), 10.70 (s, 1 H), 8.65 (d, J = 2.5 Hz, 1 H), 8.49 (dd, J = 9.0, 2.5 Hz, 1 H), 8.41 (d, J = 9.4 Hz, 1 H), 7.91 (br s, 1 H), 7.18−7.25 (m, 3 H), 7.12 (d, J = 6.6 Hz, 2 H), 7.04 (d, J = 9.2 Hz, 1 H), 6.34 (d, J = 7.2 Hz, 1 H), 5.38 (appar. quartet, J = 6.8 Hz, 1 H), 4.61 (appar. t, J = 8.5 Hz, 1 H), 4.04 (s, 3 H), 3.35 (appar. sextet, J = 6.8 Hz, 1 H), 3.23 (dd, J = 13.7, 6.3 Hz, 1 H), 3.08−3.16 (m, 2 H), 2.40 (appar. septet, J = 6.9 Hz, 1 H), 2.17 (septet, J = 7.0 Hz, 1 H), 1.51 (appar. pentet, J = 7.3 Hz, 2 H), 1.35 (sextet, J = 7.4 Hz, 2 H), 1.14 (d, J = 6.9 Hz, 3 H), 1.13 (d, J = 6.8 Hz, 3 H), 1.06 (d, J = 6.6 Hz, 3 H), 1.05 (d, J = 6.7 Hz, 3 H), 0.90 (t, J = 7.3 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.7, 169.8, 165.1, 159.8, 158.8, 157.8, 154.2, 135.5, 131.6, 129.5, 128.4, 127.1, 126.4, 124.5, 119.1, 111.8, 59.2, 56.6, 51.4, 39.9, 39.2, 35.5, 31.9, 31.6, 20.2, 19.7, 19.2, 19.0, 13.8; HRMS (SIMS) m/z for $C_{32}H_{44}N_6O_7Na$ $[M + Na]^+$ Calcd 647.3169, found 647.3195. A sample of **9**, prepared by solid-phase synthesis on indole resin,[25] was subjected to elemental analysis:

Anal. Calcd for $C_{32}H_{44}N_6O_7$: C, 61.52; H, 7.10; N, 13.45. Found: C, 61.39; H, 7.12; N, 13.28.

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