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Role of Secondary Structure in the Asymmetric Acylation Reaction Catalyzed by Peptides Based on Chiral C^{α}-Tetrasubstituted α-Amino Acids

Fernando Formaggio,[†] Alessandra Barazza,[†] Andrea Bertocco,[†] Claudio Toniolo,*[,†] Quirinus B. Broxterman,[‡] Bernard Kaptein,[‡] Elena Brasola,[§] Paolo Pengo,[§] Lucia Pasquato,[§,||] and Paolo Scrimin[§]

Department of Chemistry, University of Padova, and ICB, CNR, 35131 Padova, Italy, DSM Pharma Chemicals, Advanced Synthesis and Catalysis, P.O. Box 18, 6160 MD Geleen, The Netherlands, and Department of Chemistry, University of Padova, and ITM, CNR, 35131 Padova, Italy

claudio.toniolo@unipd.it

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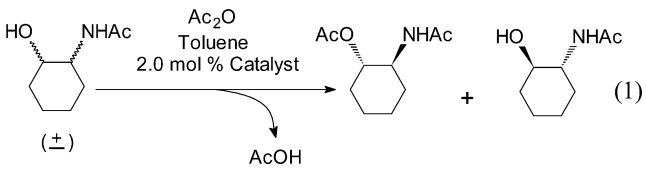
In a recent series of papers, Miller and co-workers were able to show that His(π-Me)-based, terminally protected peptides are potent catalysts of the asymmetric acyl transfer reaction, useful for the kinetic resolution of alcohols. In a structure-supporting solvent, one of the most active compounds, an Aib-containing tetrapeptide, is folded in a doubly intramolecularly H-bonded β-hairpin motif incorporating a type-II′ β-turn conformation. In this work, we have expanded the study of the Miller tetrapeptide by examining a set of analogues and shorter sequences (dipeptide amides), characterized by chiral C^{α}-tetrasubstituted α-amino acids of diverging bulkiness and optical configuration. Peptide synthesis in solution, conformational analysis by FT-IR absorption and ^{1}H NMR techniques, and screening of catalytic activity as well have been performed. Our results confirm the close relationship between the β-hairpin 3D-structure and the catalytic activity of the peptides. A tetrapeptide analogue slightly more selective than the Miller compound has been found. However, the terminally protected, industrially more appealing, dipeptide amides are poorly effective.

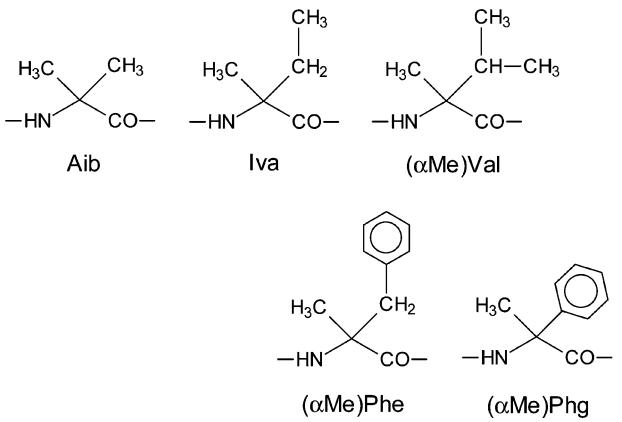
Introduction

Significant improvements were recently achieved in the development of peptide-based, chiral, nucleophilic catalysts for the kinetic resolution of racemic alcohols by means of enantioselective acyl transfer reactions (eq 1).[1]



(1)

A large body of results from Miller's laboratory[2] clearly demonstrated that terminally protected, His(π-Me)-based peptides as small as tripeptide amides and tetra-, penta-, and octapeptide esters can be effectively exploited for this purpose. From conformational investigations it was proposed that rigidified β-hairpin-forming peptide platforms are an absolute prerequisite for significant catalytic activity and enantioselectivity. These catalysts, active as monomers, show enantioselective rate accelerations consistent with a preferential transition-state stabilization governed by catalyst-substrate secondary contacts.



We focused on the Miller's prototypical, terminally protected, tetrapeptide catalyst Boc-L-His(π-Me)-D-Pro-Aib-L-Phe-OMe **1′**, which causes the reaction of the (*R*,*R*) alcohol enantiomer (eq 1) to proceed remarkably faster than that of the (*S*,*S*) enantiomer (k_{rel} = 28).[2a,d] In a structure-supporting solvent this peptide adopts a β-hairpin conformation characterized by [His(π-Me)]C═O···H−N(Phe) and [His(π-Me)]N−H···O═C(Phe) intramolecular H-bonds. The onset of a type-II′ β-turn[3] in the -D-Pro-Aib- sequence of this compound is not unexpected, as it is known to typically occur in a -D-L-amino acid stereo-

* To whom correspondence should be addressed.
† University of Padova, ICB.
‡ DSM Pharma Chemicals.
§ University of Padova, ITM.
|| Present address: Department of Chemical Sciences, University of Trieste, 34127 Trieste, Italy.

(1) (a) Hoveyda, A. H. *Chem. Biol.* **1999**, *6*, R305−R308. (b) Jarvo, E. R.; Miller, S. J. *Tetrahedron* **2002**, *58*, 2481−2495.

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sequence[3] (Aib, being achiral, can explore backbone torsion angles appropriate for either a D- or an L-residue).

The aim of the present investigation was 2-fold: (i) As subtle perturbations in the sequence of these small peptide catalysts were shown to lead to dramatic differences in enantioselectivity,[1] we decided to synthesize and compare the conformational and catalytic properties of compound **1′** with those of its tetrapeptide analogues **2**−**4** characterized at position 3 of the sequence by an Aib → L-Iva (**2**) or an Aib → L-(αMe)Val (**3**) replacement or at position 4 of the sequence by an L-Phe → L-(αMe)Phg (**4**) replacement, respectively. (ii) As extremely short peptides (e.g., dipeptides) are particularly attractive for industrial applications, we also prepared and examined the terminally protected dipeptide amides **5**−**10** (still long enough to fold in an intramolecularly H-bonded *β*-turn conformation) by preserving the nucleophilic His(*π*-Me) residue at position 1 followed by either an L- or a D-(αMe)Val, (αMe)Phe or (αMe)Phg residue.

Iva, (αMe)Val, (αMe)Phe, and (αMe)Phg are C^{α}-tetrasubstituted, chiral α-amino acids, members of the same family the parent compound of which is Aib.[4] They all share with Aib the propensity for helical backbone torsion angles,[4d,e,5] appropriate for the $i + 2$ corner of a (more or less distorted) *β*-turn. However, while L-(αMe)Val exhibits an extremely strong bias for right-handed helical torsion angles, L-Iva, L-(αMe)Phe, and L-(αMe)Phg were more or less frequently shown to fold in the left-handed helical structure.

Results and Discussion

Peptide Synthesis and Characterization. For the large-scale production of the enantiomerically pure L-Iva, L- and D-(αMe)Val, L- and D-(αMe)Phe, and L- and D-(αMe)Phg an economically attractive and generally applicable chemo-enzymatic synthesis was developed by the DSM group a few years ago.[6] It involves a combination of organic synthesis for the preparation of the racemic α-amino amide followed by the use of a broadly specific α-amino amidase to achieve enantioselective resolution.

The Cbz N^{α}-protected α-amino acids were obtained by reacting the free amino acid with the Cbz 1-oxysuccinimido ester[7] in acetonitrile/water in the presence of triethylamine (TEA) or in acetonitrile in the presence of tetramethylammonium hydroxide. In the difficult coupling steps between two sterically demanding residues the EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide] method with the efficient additive HOAt (7-aza-1-hydroxy-1,2,3-benzotriazole)[8] in methylene chloride in the presence of NMM (*N*-methylmorpholine) was used. The reaction yields ranged from moderate to good. Removal of the Cbz N^{α}-protecting group was performed by catalytic hydrogenation. Boc-L-His(*π*-Me)-OH was a commercially available product. Before use we have verified its identity and purity by mono- and bidimensional NMR experiments.[9]

Initially, we discovered a substantial discrepancy between the ^{1}H NMR spectra of tetrapeptides **1** and **1′**. It involved the signal of the characteristic CH^2 proton of the His(*π*-Me) imidazole ring, which for **1** was seen at 8.48 ppm in $CDCl_3$ solution, whereas it was reported at 7.40 for **1′** [2a,d] (and tested on an authentic sample kindly provided by Dr. S. J. Miller). It turned out that the factor responsible for this anomalous behavior was the protonation of the imidazole moiety that took place during our synthetic protocol (peptide **1**). Indeed, after deprotonation of peptide **1** by treatment with TEA, its NMR spectrum matched well that published for **1′**. Therefore, before testing the catalytic activity of our peptides **1**−**10**, all of them were treated with 3 equiv of TEA for 20 min, the solutions were evaporated several times with acetonitrile, and the peptides were liophilized.

Solution Conformational Analysis. Among the tetrapeptides with a replacement at position 3 we have investigated in detail the preferred conformation of the most sterically hindered, prototypical, L-(αMe)Val-containing **3** in the structure supporting solvent $CDCl_3$ by FT-IR absorption and ^{1}H NMR techniques. In the FT-IR absorption spectrum (amide A region) two intense bands are seen (Figure 1A). From an inspection of the relative areas of the free (3437 cm^{-1}) to the broad H-bonded (3363−3355 cm^{-1}) N−H stretching band[10] it is evident that two NH groups of the peptide are H-bonded while only one NH group is free (as the overall spectral pattern does not change upon variation of peptide concentration from 1.0 to 0.1 mM, we can safely assume that these H-bonds are of the intramolecular type).

(2) (a) Miller, S. J.; Copeland, G. T.; Papaioannou, N.; Horstmann, T. E.; Ruel, E. M. *J. Am. Chem. Soc.* **1998**, *120*, 1629−1630. (b) Copeland, G. T.; Jarvo, E. R.; Miller, S. J. *J. Org. Chem.* **1998**, *63*, 6784−6785. (c) Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 4306−4307. (d) Jarvo, E. R.; Copeland, G. T.; Papaioannou, N.; Bonitatebus, P. J., Jr.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 11638−11643. (e) Harris, R. F.; Nation, A. J.; Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **2000**, *122*, 11270−11271. (f) Blank, J. T.; Guerin, D. J.; Miller, S. J. *Org. Lett.* **2000**, *2*, 1247−1249. (g) Jarvo, E. R.; Vasbinder, M. M.; Miller, S. J. *Tetrahedron* **2000**, *56*, 9773−9779. (h) Vasbinder, M. M.; Jarvo, E. R.; Miller, S. J. *Angew. Chem., Int. Ed.* **2001**, *40*, 2824−2827. (i) Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 6496−6502. (j) Jarvo, E. R.; Evans, C. A.; Copeland, G. T.; Miller, S. J. *J. Org. Chem.* **2001**, *66*, 5522−5527.

(3) (a) Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425−1436. (b) Toniolo, C. *C. R. C. Crit. Rev. Biochem.* **1980**, *9*, 1−44. (c) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1−109. (d) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 6975−6985.

(4) (a) Marshall, G. R. In *Intra-Science Chemistry Report*; Kharasch, N., Ed.; Gordon and Breach: New York, 1971; pp 305−316. (b) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747−6756. (c) Toniolo, C.; Benedetti, E. *Macromolecules* **1991**, *24*, 4004−4009. (d) Toniolo, C.; Crisma, M.; Formaggio, F.; Valle, G.; Cavicchioni, G.; Précigoux, G.; Aubry, A.; Kamphuis, J. *Biopolymers* **1993**, *33*, 1061−1072. (e) Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C. *Biopolymers (Pept. Sci.)* **2001**, *60*, 396−419.

(5) Mossel, E.; Formaggio, F.; Valle, G.; Crisma, M.; Toniolo, C.; Doi, M.; Ishida, T.; Broxterman, Q. B.; Kamphuis, J. *Lett. Pept. Sci.* **1998**, *5*, 223−225.

(6) (a) Kruizinga, W. H.; Bolster, J.; Kellogg, R. M.; Kamphuis, J.; Boesten, W. H. J.; Meijer, E. M.; Schoemaker, H. E. *J. Org. Chem.* **1988**, *53*, 1826−1827. (b) Sonke, T.; Kaptein, B.; Boesten, W. H. J.; Broxterman, Q. B.; Kamphuis, J.; Formaggio, F.; Toniolo, C.; Rutjes, F. P. J. T.; Schoemaker, H. E. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Dekker: New York, 2000; pp 23−58.

(7) Frankel, M.; Ladkany, D.; Gilon, C.; Wolman, Y. *Tetrahedron Lett.* **1966**, *7*, 4765−4768.

(8) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.

(9) Serdijn, J.; Bloemhoff, W.; Kerling, K. E. T.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1984**, *103*, 50−54.

(10) (a) Mizushima, S.; Shimanouchi, T.; Tsuboi, M.; Souda, R. *J. Am. Chem. Soc.* **1952**, *74*, 270−271. (b) Cung, M. T.; Marraud, M.; Néel, J. *Ann. Chim. (Paris)* **1972**, *7*, 183−209. (c) Palumbo, M.; Da Rin, S.; Bonora, G. M.; Toniolo, C. *Makromol. Chem.* **1976**, *177*, 1477−1492. (d) Pulla Rao, C.; Nagaraj, R.; Rao, C. N. R.; Balaram, P. *Biochemistry* **1980**, *19*, 425−431. (e) Bonora, G. M.; Mapelli, C.; Toniolo, C.; Wilkening, R. R.; Stevens, E. S. *Int. J. Biol. Macromol.* **1984**, *6*, 179−188.

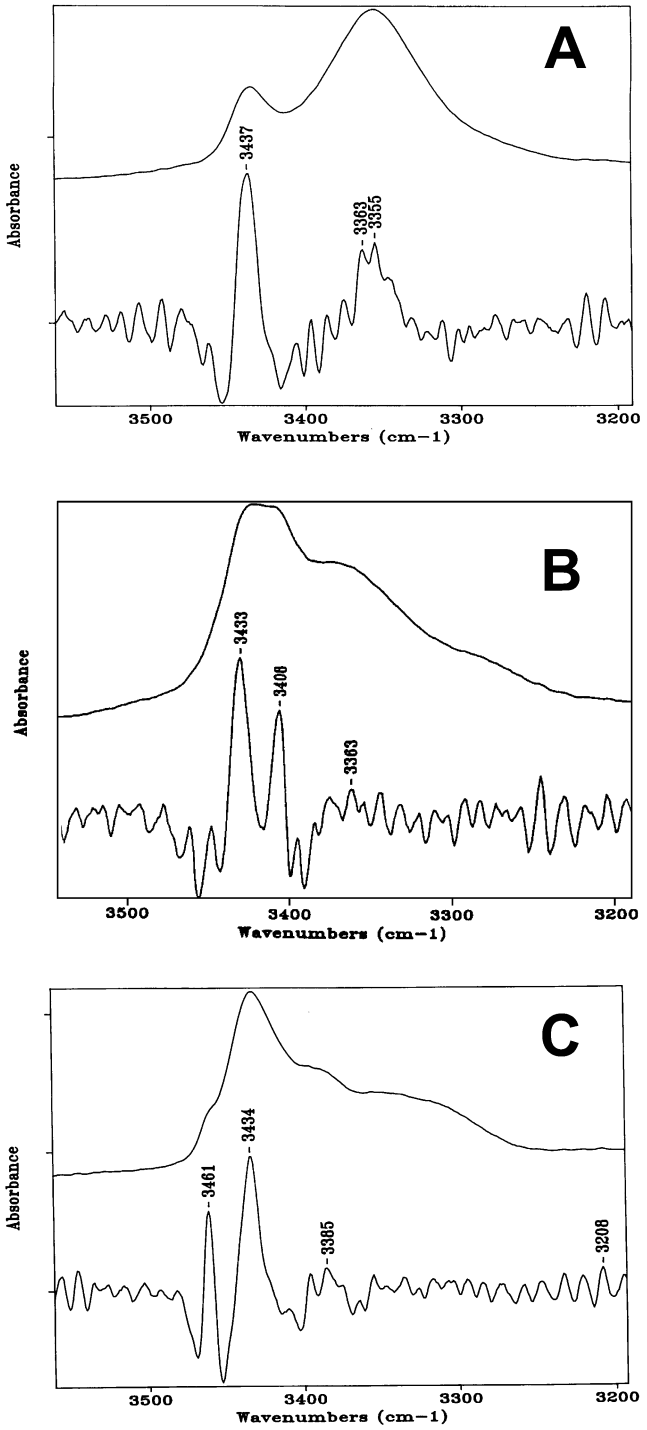

FIGURE 1. (Top) FT-IR absorption spectra (3500−3200 cm^{-1} region) and (bottom) inverted second derivative spectra of peptides **3** (A), **4** (B), and **6** (C) in $CDCl_3$ solution (concentration: 1 mM).

To obtain more detailed information on the conformational preference of **3** in $CDCl_3$ solution we performed a 400 MHz ^1H NMR analysis. The delineation of inaccessible (or intramolecularly H-bonded) NH groups was carried out by monitoring the solvent dependence of NH proton chemical shifts upon adding increasing amount of the H-bonding acceptor solvent DMSO to the $CDCl_3$ solution.[11] In the titration experiment (Figure 2) two types of NH protons were observed: type i includes the

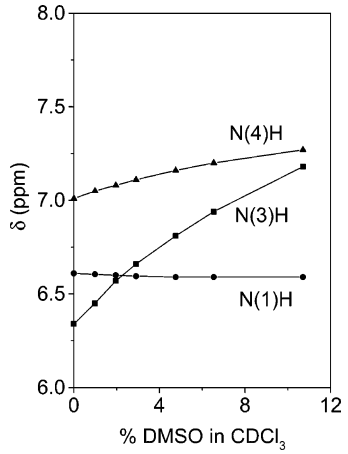

FIGURE 2. Plot of NH proton chemical shifts in the ^1H NMR spectra of peptide **3** as a function of increasing percentages of DMSO (v/v) added to the $CDCl_3$ solution (concentration: 1 mM).

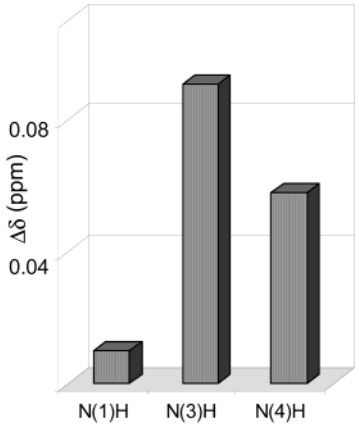

FIGURE 3. Histogram showing the NH proton chemical shift variations in the ^1H NMR spectrum of peptide **3** as a function of concentration (from 10 to 1 mM) in $CDCl_3$ solution.

(αMe)Val N(3)H proton which exhibits a remarkable downfield shift upon addition of DMSO; type ii includes the [His(π-Me)]N(1)H and Phe N(4)H protons displaying a behavior typical of shielded protons (absence or modest sensitivity of chemical shift to solvent composition). In Figure 3, the shifts experienced by the NH protons of peptide **3** upon dilution from 10 to 1 mM concentration are reported. Again, the chemical shift of the (αMe)Val N(3)H proton is the most sensitive to the environmental effect.

Taken together, these ^1H NMR results agree well with the FT-IR absorption data discussed above, allowing us to conclude that in $CDCl_3$ solution the terminally protected tetrapeptide **3** is highly folded in a conformation stabilized by two intramolecular H-bonds involving the His N(1)H and Phe N(3)H groups as donors. Interestingly, the His N(1)H proton resonates at a unusually low field (6.6 ppm) for an urethane NH proton in $CDCl_3$. This finding is in full agreement with the proposed involvement of this NH group in a H-bond. In addition, the partial ROESY spectrum shown in Figure 4 clearly

(11) (a) Kopple, K. D.; Ohnishi, M.; Go, A. *Biochemistry* **1969**, *8*, 4087−4095. (b) Martin, D.; Hauthal, H. G. *Dimethyl Sulphoxide*; Van Nostrand Reinhold: Wokingham, U.K., 1975.

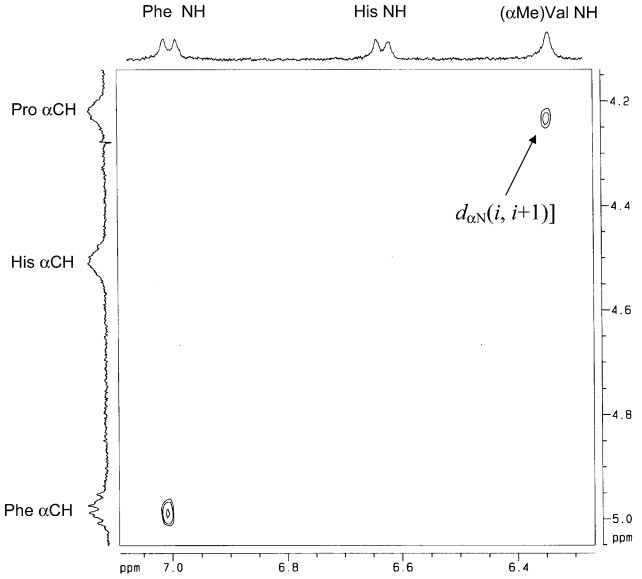

FIGURE 4. Section of the ROESY spectrum of peptide **3** in $CDCl_3$ solution (concentration 1 mM). The arrow indicates the [$d_{\alpha N}(i, i + 1)$] cross-peak typical of a type-II′ β-turn conformation.

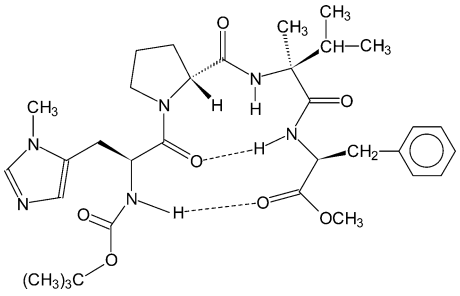

FIGURE 5. Preferred conformation of peptide **3** in $CDCl_3$ solution.

demonstrates the occurrence of a NOE interaction between the D-Pro αC(2)H and the (αMe)Val N(3)H protons, strongly indicative of a type-II′ β-turn conformation. On the basis of all these data, we therefore propose for tetrapeptide **3** the β-hairpin structure depicted in Figure 5. It is gratifying that our conformational conclusions match beautifully those published by Miller and coworkers for the Aib[3] parent tetrapeptide **1′**.[2a,d]

We have also extended our FT-IR absorption conformational analysis in $CDCl_3$ solution to tetrapeptide **4**, with a replacement at *position 4* (Figure 1B), and to the terminally protected dipeptide amides **5**−**10** (for a representative example, the D-(αMe)Val[2] dipeptide amide **6**, see Figure 1C). It is evident that in the L-(αMe)Phg[4] tetrapeptide **4** as well as in the very short dipeptides, despite the presence in the latter of a C^{α}-tetrasubstituted α-amino acid at position 2, the population of intramolecularly H-bonded conformers is remarkably smaller than that in tetrapeptide **3**.

In addition, the results of our mono- and two-dimensional NMR analyses (in particular, the positions of the NH proton chemical shifts and their variations upon DMSO addition, and the NOE effects) on tetrapeptide **4** (not shown) unambiguously indicate that the intramolecularly H-bonded type II′ β-turn conformer still forms

CHART 1

Boc-L-His(π-Me)-D-Pro-Aib-L-Phe-OMe	**1** (synthesized in our laboratory)
Boc-L-His(π-Me)-D-Pro-Aib-L-Phe-OMe	**1'** (synthesized in Miller's laboratory)
Boc-L-His(π-Me)-D-Pro-L-Iva-L-Phe-OMe	**2**
Boc-L-His(π-Me)-D-Pro-L-(αMe)Val-L-Phe-OMe	**3**
Boc-L-His(π-Me)-D-Pro-Aib-L-(αMe)Phg-OMe	**4**
Boc-L-His(π-Me)-L-(αMe)Val-NH*t*-Bu	**5**
Boc-L-His(π-Me)-D-(αMe)Val-NH*t*-Bu	**6**
Boc-L-His(π-Me)-L-(αMe)Phe-NH*t*-Bu	**7**
Boc-L-His(π-Me)-D-(αMe)Phe-NH*t*-Bu	**8**
Boc-L-His(π-Me)-L-(αMe)Phg-NH*t*-Bu	**9**
Boc-L-His(π-Me)-D-(αMe)Phg-NH*t*-Bu	**10**

to a remarkable extent, but the occurrence of the additional intramolecular H-bond stabilizing the β-hairpin platform is much reduced.

Tetra- and Dipeptides as Enantioselective Acylation Catalysts. The peptides reported in Chart 1 were used as catalysts in the model reaction (eq 1) using 5 mol % of catalyst and 8.8 equiv of acetic anhydride in toluene at 25 °C. The reaction course was monitored using GC by sampling the reaction mixture at appropriate time intervals. Each sample was quenched with methanol, and subsequently, the extent of conversion, i.e. the concentrations of products (both enantiomers) and reagents (both enantiomers), was determined. The enantioselections were calculated according to the method of Kagan[12] ($k_{rel} = k_{(R,R)}/k_{(S,S)}$). As the reactions are pseudo-first-order, the ratio between the rate constants as well as the enantiomeric excess do not depend on the extent of conversion. Evaluation of racemic 2-acetamidocyclohexanol in the asymmetric acetylation reaction in the presence of the terminally protected tetrapeptide ester catalyst **1** (synthesized in our laboratory) showed an excellent enantioselection ($k_{rel} = 28$) (Table 1), thereby exactly reproducing the published results of Miller et al.[2a,d] In our hands the same peptide prepared in Miller's laboratory and shipped to us (**1′**) exhibited a modestly lower stereodifferentiation ($k_{rel} = 22$). The two other tetrapeptides examined in this work with replacement at position 3 (**2** and **3**) are both significantly active. In particular, replacement of the Aib[3] residue with L-Iva (analogue **2**), with its linear but bulkier side chain, is characterized by a selectivity ($k_{rel} = 23$) slightly decreased as compared to that of **1**. In addition, the enhanced steric hindrance slows down significantly (35−45%) the transacylation reaction of both substrate enantiomers. The negative reactivity trend is even more marked with analogue **3**, with its bulkier, β-branched L-(αMe)Val residue at position 3. Interestingly, however, this analogue is more, although not dramatically, selective ($k_{rel} = 33$) than the Aib-containing prototypical tetrapeptide **1**.

As compared to **1**, replacement of the proteinogenic L-Phe with the C^{α}-tetrasubstituted L-(αMe)Phg residue at position 4 (tetrapeptide **4**) is accompanied not only by a significantly decreased selectivity ($k_{rel} = 10$) but by a reduced reactivity as well. These results, together with the decreased population of the double intramolecularly H-bonded conformation for this peptide (see above), indicate that the overall conformational constraint plays a key role in the enantioselectivity of the catalyst.

(12) Kagan, H. B.; Fiand, J. C. *Top. Stereochem.* **1988**, *18*, 249−330.

TABLE 1. Selectivities in the Kinetic Resolutions of the Secondary Alcohol (±)-2-Acetamidocyclohexanol with Catalysts 1−10

	catalyst	$k_{(R,R)}$ (min^{-1})[a]	$k_{(S,S)}$ (min^{-1})[a]	$k_{(R,R)}/k_{(S,S)}$ (k_{rel})
1′	Boc-L-His(π-Me)-D-Pro-Aib-L-Phe-OMe[b]	0.0470	0.0021	22
1	Boc-L-His(π-Me)-D-Pro-Aib-L-Phe-OMe[c]	0.0334	0.0012	28
2	Boc-L-His(π-Me)-D-Pro-L-Iva-L-Phe-OMe	0.0184	0.0008	23
3	Boc-L-His(π-Me)-D-Pro-L-(αMe)Val-L-Phe-OMe	0.0098	0.0003	33
4	Boc-L-His(π-Me)-D-Pro-Aib-L-(αMe)Phg-OMe	0.0142	0.0014	10
5	Boc-L-His(π-Me)-L-(αMe)Val-NH*t*-Bu	0.0045	0.0019	2.3
6	Boc-L-His(π-Me)-D-(αMe)Val-NH*t*-Bu	0.0018	0.0036	0.5
7	Boc-L-His(π-Me)-L-(αMe)Phe-NH*t*-Bu	0.0035	0.0029	1.2
8	Boc-L-His(π-Me)-D-(αMe)Phe-NH*t*-Bu	0.0028	0.0041	0.7
9	Boc-L-His(π-Me)-L-(αMe)Phg-NH*t*-Bu	0.0025	0.0019	1.3
10	Boc-L-His(π-Me)-D-(αMe)Phg-NH*t*-Bu	0.0034	0.0037	0.9

[a] Average values of two or three independent experiments. [b] Peptide synthesized in Dr. S. J. Miller's laboratory. [c] Peptide synthesized in our laboratory.

Finally, all terminally protected dipeptide amides investigated (**5**−**10**) (Table 1) are very poorly efficient as catalysts. Even more important is the observation that all of them exhibit an extremely modest enantiomeric selectivity. However, it is worth noting that all sets of diastereomeric peptides (**5** vs **6**; **7** vs **8**; **9** vs **10**) show opposite enantioselectivities.

Conclusion

Incorporation of noncoded, C^{α}-tetrasubstituted α-amino acids[4] into peptides drastically reduces their conformational freedom by stabilizing specific secondary structures such as β-turns,[3] and 3_{10}- and α-helices.[13] Peptides based on this class of residues may represent unique foldamers[14] and can be exploited as rigidified molecular spacers and templates for the design of mini-receptors, spectroscopic and electrochemical molecular rulers, and catalysts.[15]

Miller and co-workers[2] recently expanded this field by showing that terminally protected peptides can be used as catalysts for the kinetic resolution of racemic alcohols. As most of these peptides are characterized by an Aib residue, the prototype of C^{α}-tetrasubstituted α-amino acids,[4] they are strongly biased toward a β-turn conformation in solution which decreases catalyst flexibility. Indeed, all of the tripeptide amides and the tetra- and pentapeptide esters examined showing catalytic activity share the common feature of a β-hairpin conformation, in which a type-II′ β-turn, generated by a -D-Pro-Aib-(i + 1, i + 2) sequence, is further stabilized by an (i) N−H···O=C(i + 3) intramolecular H-bond. These authors also demonstrated that: (i) the Aib residue at position i + 2 of the β-turn can be replaced by other *achiral* members of its class, e.g. $C^{\alpha,\alpha}$-di-*n*-butylglycine or 1-aminocyclohexane-1-carboxylic acid, with only a limited detrimental effect on resolution efficiency,[2j] and (ii) the Phe residue at position i + 3 of the β-turn cannot be replaced by the C^{α}-tetrasubstituted residues 1-aminocyclopentane-1-carboxylic acid or 1-aminocyclohexane-1-carboxylic acid without an almost complete loss of enantioselectivity.[2j]

In the present work, we have further extended the investigation on the Miller tetrapeptide **1′** by synthesizing and studying two tetrapeptide analogues (**2** and **3**) with the *achiral* Aib residue replaced by a *chiral* amino acid of its class with different side-chain bulkiness and helical screw-sense propensity [L-(αMe)Val is remarkably bulkier and more strongly biased toward the right-handed helical structure, appropriate for the i + 2 position of a type-II′ β-turn, than L-Iva].[4d,e] We found that the transacylation reactivity is very sensitive to the steric hindrance of the residue at position i + 2, rapidly decreasing from Aib to Iva and from Iva to (αMe)Val. However, both analogues are remarkably stereoselective with the L-(αMe)Val-based peptide catalyst **3** being even more effective than **1** (or **1′**). Also, our conformational analysis by FT-IR absorption and ^{1}H NMR lent credence to the close correlation between catalyst stereoselection efficiency and a β-hairpin, rigidified structure (for analogue **3** see Figure 5), as proposed by Miller and co-workers.[2]

In the capture of the acylating reagent by the His(π-Me) nucleophilic side chain these authors invoked the occurrence of two diastereomeric transition states of diverging stability on the basis of differential H-bonding interactions of alcohol substrate enantiomers with the chiral environment generated by the highly folded tetrapeptide catalyst.[2a,d] In this connection, the important role played by the Pro-Xxx secondary amide bond was also demonstrated.[2h] Our findings on tetrapeptides **1**−**3** fit nicely into this reaction scheme and unambiguously confirm that β-turn formation (and its stabilization via β-hairpin) in the peptide catalyst is a prerequisite for excellent reactivity and stereoselectivity and that enhanced steric hindrance at position i+2 of the β-turn is not detrimental, or it might be even beneficial, for the stereoselection governed by the ancillary H-bonding

(13) (a) Toniolo, C.; Benedetti, E. *Trends Biochem. Sci.* **1991**, *16*, 350−353. (b) Bolin, K. A.; Millhauser, G. L. *Acc. Chem. Res.* **1999**, *32*, 1027−1033. (c) Pal, L.; Basu, G.; Chakrabarti, P. *Proteins: Struct. Funct. Genet.* **2002**, *48*, 571−579.

(14) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180.

(15) (a) Scarso, A.; Scheffer, U.; Göbel, M.; Broxterman, Q. B.; Kaptein, B.; Formaggio, F.; Toniolo, C.; Scrimin, P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5144−5149. (b) Formaggio, F.; Bonchio, M.; Crisma, M.; Peggion, C.; Mezzato, S.; Polese, A.; Barazza, A.; Antonello, S.; Maran, F.; Broxterman, Q. B.; Kaptein, B.; Kamphuis, J.; Vitale, R. M.; Saviano, M.; Benedetti, E.; Toniolo, C. *Chem. Eur. J.* **2002**, *8*, 85−93. (c) Bianco, A.; Corvaja, C.; Crisma, M.; Guldi, D. M.; Maggini, M.; Sartori, E.; Toniolo, C. *Chem. Eur. J.* **2002**, *8*, 1544−1553. (d) Pispisa, B.; Palleschi, A.; Stella, L.; Venanzi, M.; Mazzuca, C.; Formaggio, F.; Toniolo, C.; Broxterman, Q. B. *J. Phys. Chem.* **2002**, *B106*, 5733−5738. (e) Hanson, P.; Millhauser, G. L.; Formaggio, F.; Crisma, M.; Toniolo, C. *J. Am. Chem. Soc.* **1996**, *118*, 7618−7625. (f) Antonello, S.; Formaggio, F.; Moretto, A.; Toniolo, C.; Maran, F. *J. Am. Chem. Soc.* **2003**, *125*, 2874−2875. (g) Voyer, N.; Lamothe, J. *Tetrahedron* **1995**, *51*, 9241−9284. (h) Andrews, M. J. I.; Tabor, A. B. *Tetrahedron* **1999**, *55*, 11711−11743.

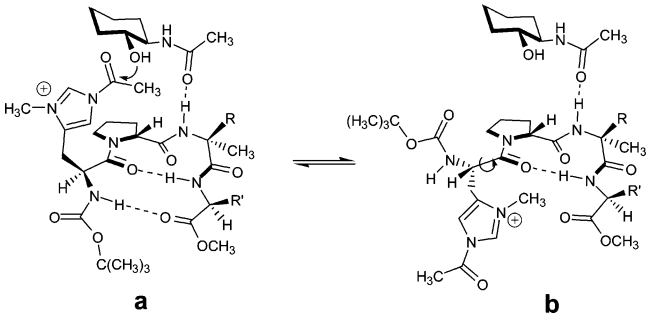

FIGURE 6. Transition state proposed for the fast reacting enantiomer (**a**) and the conformer obtained by rotation of the $C^{\alpha}-C'$ bond of the L-His(π-Me) residue in the presence of a weaker H-bonding motif (**b**).

interaction between the peptide catalyst and the racemic alcohol substrate (Figure 6a).

Furthermore, our results on tetrapeptide **4** indirectly support the contention that a β-hairpin template (not just a β-turn template) is strictly required for a peptide catalyst to exhibit significantly different rates in the acylation of the alcohol enantiomers. Indeed, as already reported by Jarvo et al.,[2j] incorporation at position 4 of the β-turn of a C^{α}-tetrasubstituted α-amino acid, e.g. L-(αMe)Phg, with its propensity for a more or less extended structure, weaker than that of L-Phe,[4e,5] seems to reduce the population of conformers with the [His(π-Me)]N−H···O═C(Phe) intramolecular H-bond, thereby enhancing their conformational freedom and concomitantly decreasing peptide catalyst stereoselectivity. A weaker H-bonding stabilizing motif implies the existence of an equilibrium where a significant population of a conformer (Figure 6b) is present in which the catalytic subunit does not reside on the same face of the recognition site for the substrate.

In search of a shorter, industrially more attractive, peptide catalyst of this asymmetric acylation reaction we have also synthesized and investigated the terminally protected dipeptide amides **5**−**10**. In these compounds, long enough to generate an intramolecularly H-bonded β-turn but too short for β-hairpin formation, the nucleophilic His(π-Me) residue at position 1 is followed by a chiral C^{α}-tetrasubstituted α-amino acid. Again, we have noted a close relationship between peptide catalyst conformation and stereoselectivity in that, unfortunately, the population of β-turn conformers is quite limited in these dipeptide amides and their stereoselectivity is poor.

Taken together, our experimental findings underscore the points that a rigid, β-hairpin secondary structure is an element of paramount importance characterizing the platform required by a peptide to be an efficient stereoselective catalyst and that, in this connection, chiral C^{α}-tetrasubstituted α-amino acids may play a fundamental role.

Experimental Section

General Procedures. General methods for the synthesis of peptides containing C^{α}-tetrasubstituted α-amino acids were as described previously.[16] High-resolution mass spectra were obtained by electrospray ionization (ESI) on a Perseptive Biosystems Mariner API-TOF spectrometer; a 1 nM solution of neurotensin, angiotensin I and bradykinin in acetonitrile/water 1:1 mixture, containing 1% formic acid, was used for calibration. Melting points are uncorrected. Analytical thin-layer chromatography (TLC) was performed by using silica gel plates in the following solvent systems: (I) chloroform−EtOH 9:1; (II) 1-BuOH−AcOH−water 6:2:2; (III) toluene−EtOH 7:1. The compounds were revealed either with the aid of a UV lamp or with the hypochlorite-starch-iodide chromatic reaction (a single spot was observed in each case). The solid-state FT-IR absorption spectra were recorded in KBr pellets. Unless otherwise specified, all ^{1}H NMR spectra were recorded at 250 MHz in $CDCl_3$ or in $CDCl_3$−DMSO-d_6 solutions. Chemical shifts are reported in ppm (δ units) downfield of internal TMS.

General Procedure for the Synthesis of N^{α}-Protected α-Aminoacyl *tert*-Butylamides. To a solution of the Cbz N^{α}-protected α-amino acid (4.15 mmol) in CH_2Cl_2 (30 mL), cooled to 0 °C, was added EDC·HCl (4.18 mmol). After 15 min, NH_2 *t*-Bu (20.9 mmol) was added, and the solution was stirred at rt for 4 d. Then, the solvent was removed in vacuo and the residue dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, water, 5% $NaHCO_3$, and water, dried over Na_2SO_4, and evaporated to dryness. The product was crystallized from EtOAc−PE (petroleum ether).

Cbz-L-(αMe)Val-NH *t*-Bu: yield 81%; mp 116−117 °C; $[\alpha]^{20}_D$ 8.1 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.50; IR (KBr) ν_{max} 3418, 3299, 1719, 1660, 1536 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.34 (5H, m), 6.26 (1H, s), 5.33 (1H, s), 5.09 (2H, m), 2.30 (1H, m), 1.42 (3H, s), 1.30 (9H, s), 0.91 (6H, m); MS (ESI-TOF) *m*/*z* calcd for $C_{18}H_{29}N_2O_3$ 321.2173, found 321.2210 $[M + H]^+$.

Cbz-D-(αMe)Val-NH *t*-Bu: yield 85%; mp 115−117 °C; $[\alpha]^{20}_D$ −8.1 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.50; IR (KBr) ν_{max} 3418, 3299, 1718, 1659, 1535 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.34 (5H, m), 6.26 (1H, s), 5.32 (1H, s), 5.09 (2H, m), 2.29 (1H, m), 1.42 (3H, s), 1.30 (9H, s), 0.91 (6H, m); MS (ESI-TOF) *m*/*z* calcd for $C_{18}H_{29}N_2O_3$ 321.2173, found 321.2201 $[M + H]^+$.

Cbz-L-(αMe)Phe-NH *t*-Bu: yield 63%; mp 119−120 °C; $[\alpha]^{20}_D$ −51.2 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.65; IR (KBr) ν_{max} 3282, 1722, 1664, 1658, 1542, 1514 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.40−7.08 (10H, m), 5.83 (1H, s), 5.27 (1H, s), 5.14 (2H, s), 3.27 (1H, d, *J* 13.8 Hz), 3.13 (1H, d, *J* 13.8 Hz), 1.48 (3H, s), 1.31 (9H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{22}H_{29}N_2O_3$ 369.2173, found 369.2201 $[M + H]^+$.

Cbz-D-(αMe)Phe-NH *t*-Bu: yield 68%; mp 120−121 °C; $[\alpha]^{20}_D$ 52.8 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.65; IR (KBr) ν_{max} 3280, 1721, 1659, 1540, 1516 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.36−7.07 (10H, m), 5.82 (1H, s), 5.27 (1H, s), 5.12 (2H, s), 3.27 (1H, d, *J* 13.8 Hz), 3.13 (1H, d, *J* 13.8 Hz), 1.48 (3H, s), 1.28 (9H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{22}H_{29}N_2O_3$ 369.2173, found 369.2240 $[M + H]^+$.

Cbz-L-(αMe)Phg-NH *t*-Bu: yield 75%; mp 103−104 °C; $[\alpha]^{20}_D$ 15.8 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.65; IR (KBr) ν_{max} 3260, 1719, 1670, 1527 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.37−7.21 (10H, m), 6.57 (1H, s), 5.24 (1H, s), 4.96 (2H, m), 1.85 (3H, s), 1.16 (9H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{21}H_{27}N_2O_3$ 355.2016, found 355.2055 $[M + H]^+$.

Cbz-D-(αMe)Phg-NH *t*-Bu: yield 84%; mp 104−105 °C; $[\alpha]^{20}_D$ −14.7 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.65; IR (KBr) ν_{max} 3258, 1717, 1668, 1526 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.37−7.21 (10H, m), 6.55 (1H, s), 5.23 (1H, s), 4.96 (2H, m), 1.85 (3H, s), 1.16 (9H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{21}H_{27}N_2O_3$ 355.2016, found 355.2062 $[M + H]^+$.

General Procedure for the Synthesis of N^{α}-Protected Peptidyl Methyl Esters and *tert*-Butylamides. To a solution of Cbz (or Boc) N^{α}-protected α-amino acid (4.45 mmol) in CH_2Cl_2 (30 mL), cooled to 0 °C, were added HOAt (5.56 mmol) and EDC·HCl (5.56 mmol). After 10 min, the C-protected (or *tert*-butylamide) α-amino acid or peptide hydrochloride (5.56

(16) (a) Formaggio, F.; Broxterman, Q. B.; Toniolo, C. In *Houben-Weyl, Methods of Organic Synthesis*; Goodman, M., Felix, P., Moroder, L., Toniolo, C., Eds.; Thieme: Stuttgart, Germany, 2003; Vol. E22c, pp 292−310. (b) Peggion, C.; Fiammengo, R.; Mossel, E.; Broxterman, Q. B.; Kaptein, B.; Kamphuis, J.; Formaggio, F.; Crisma, M.; Toniolo, C. *Tetrahedron* **2000**, *56*, 3589−3601.

mmol) and NMM (11.13 mmol) were added and the solution was stirred at rt for 3 d. Then, the solvent was removed in vacuo and the residue dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, water, 5% $NaHCO_3$, and water, dried over Na_2SO_4, and evaporated to dryness.

Cbz-Aib-L-Phe-OMe (lit.[17a]): yield 89%; mp 93−94 °C (EtOAc−PE); $[\alpha]^{20}_D$ 2.0 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.65; IR (KBr) ν_{max} 3425, 1740, 1661, 1538 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35−7.07 (10H, m), 6.63 (1H, br d), 5.24 (1H, s), 5.07 (2H, s), 4.82 (1H, m), 3.71 (3H, s), 3.10 (2H, m), 1.47 (6H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{22}H_{27}N_2O_5$ 399.1914, found 399.1959 $[M + H]^+$.

Cbz-D-Pro-Aib-L-Phe-OMe: yield 71%; mp 65−70 °C (waxy solid); $[\alpha]^{20}_D$ 29.4 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.35; IR (KBr) ν_{max} 3325, 1745, 1682, 1535 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.34−7.12 (11H, m), 6.84 (1H, br d), 5.13 (2H, m), 4.80 (1H, m), 4.18 (1H, m), 3.68 (3H, s), 3.52 (2H, m), 3.11 (2H, m), 2.24−1.86 (4H, m), 1.45 (3H, s), 1.36 (3H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{27}H_{34}N_3O_6$ 496.2442, found 496.2489 $[M + H]^+$.

Boc-L-His(π-Me)-D-Pro-Aib-L-Phe-OMe (1) (lit.[2a,d]): yield 72% [after purification by flash chromatography (95:5, CH_2Cl_2−EtOH)]; mp 100−103 °C (waxy solid); $[\alpha]^{20}_D$ −28.4 (*c* 0.5, MeOH); R_f(I) 0.25, R_f(II) 0.55, R_f(III) 0.05; IR (KBr) ν_{max} 3338, 1743, 1679, 1639, 1511 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.39 (1H, s), 7.29−7.15 (7H, m), 6.69 (1H, s), 6.52 (1H, s), 6.01 (1H, d, *J* 8.0 Hz), 4.90 (1H, m), 4.54 (1H, m), 4.22 (1H, m), 3.88 (3H, s), 3.69 (3H, s), 3.61−3.51 (2H, m), 3.20−2.91 (4H, m), 2.13−2.04 (4H, m), 1.52 (3H, s), 1.41 (9H, s), 1.33 (3H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{31}H_{45}N_6O_7$ 613.3344, found 613.3303 $[M + H]^+$.

Cbz-L-Iva-L-Phe-OMe (lit.[17b]): yield 88%; mp 95−96 °C (EtOAc−PE); $[\alpha]^{20}_D$ −15.6 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.55; IR (KBr) ν_{max} 3329, 3319, 1751, 1692, 1662, 1537, 1521 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.34−7.08 (10H, m), 6.45 (1H, d, *J* 7.2 Hz), 5.56 (1H, s), 5.06 (2H, s), 4.87 (1H, m), 3.71 (3H, s), 3.11 (2H, m), 1.68 (2H, m), 1.44 (3H, s), 0.76 (3H, t, *J* 7.1 Hz); MS (ESI-TOF) *m*/*z* calcd for $C_{23}H_{29}N_2O_5$ 413.2071, found 413.2121 $[M + H]^+$.

Cbz-D-Pro-L-Iva-L-Phe-OMe: yield 89% [after purification by flash chromatography] (98:2, CH_2Cl_2−EtOH)]; mp 73−74 °C; $[\alpha]^{20}_D$ 21.8 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.40; IR (KBr) ν_{max} 3332, 1746, 1684, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.34−7.12 (10H, m), 6.94 (1H, s), 6.87 (1H, d, *J* 5.9 Hz), 5.12 (2H, s), 4.83 (1H, m), 4.22 (1H, m), 3.69 (3H, s), 3.55−3.38 (2H, m), 3.18 (1H, dd, *J* 5.7, 14.0 Hz), 3.04 (1H, dd, *J* 6.7, 14.0 Hz), 2.32−1.76 (6H, m), 1.43 (3H, s), 0.65 (3H, br t); MS (ESI-TOF) *m*/*z* calcd for $C_{28}H_{36}N_3O_6$ 510.2598, found 510.2637 $[M + H]^+$.

Boc-L-His(π-Me)-D-Pro-L-Iva-L-Phe-OMe (2): yield 61% [after purification by flash chromatography (93:7, CH_2Cl_2−EtOH)]; mp 67−68 °C; $[\alpha]^{20}_D$ −25.6 (*c* 0.5, MeOH); R_f(I) 0.40, R_f(II) 0.25, R_f(III) 0.05; IR (KBr) ν_{max} 3336, 1745, 1688, 1639, 1507 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.46 (1H, s), 7.21−7.12 (6H, m), 6.76 (1H, s), 6.69 (1H, s), 6.04 (1H, d, *J* 7.1 Hz), 4.81 (1H, m), 4.51 (1H, m), 4.04 (1H, m), 3.62 (3H, s), 3.59 (3H, s), 3.41−3.36 (2H, m), 3.14−2.75 (4H, m), 2.02−1.52 (6H, m), 1.38 (3H, s), 1.34 (9H, s), 0.58 (3H, t, *J* 7.2 Hz); MS (ESI-TOF) *m*/*z* calcd for $C_{32}H_{47}N_6O_7$ 627.3501, found 627.3548 $[M + H]^+$.

Cbz-L-(αMe)Val-L-Phe-OMe (lit.[17b]): yield 88%; mp 98−99 °C ($CHCl_3$−PE); $[\alpha]^{20}_D$ −10.0 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.55; IR (KBr) ν_{max} 3402, 3303, 1746, 1721, 1665, 1539 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.35−7.12 (10H, m), 6.85 (1H, br d), 5.22 (1H, s), 5.07 (2H, s), 4.87 (1H, m), 3.69 (3H, s), 3.15 (1H, dd, *J* 5.8, 14.3 Hz), 3.06 (1H, dd, *J* 7.1, 14.3 Hz), 2.12 (1H, m), 1.41 (3H, s), 0.87 (3H, d, *J* 6.8 Hz), 0.77 (3H, d, *J* 6.8 Hz); MS (ESI-TOF) *m*/*z* calcd for $C_{24}H_{31}N_2O_5$ 427.2227, found 427.2252 $[M + H]^+$.

Cbz-D-Pro-L-(αMe)Val-L-Phe-OMe: yield 89%; mp 85−86 °C (EtOAc−PE); $[\alpha]^{20}_D$ 23.2 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.40; IR (KBr) ν_{max} 3321, 1734, 1691, 1660, 1540 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35−7.18 (11H, m), 6.86 (1H, s), 5.13 (2H, s), 4.84 (1H, m), 4.25 (1H, m), 3.68 (3H, s), 3.67−3.49 (2H, m), 3.14 (2H, m), 2.27−1.77 (5H, m), 1.43 (3H, s), 0.74 (3H, d, *J* 6.9 Hz), 0.68 (3H, d, *J* 6.0 Hz); MS (ESI-TOF) *m*/*z* calcd for $C_{29}H_{38}N_3O_6$ 524.2756, found 524.2794 $[M + H]^+$.

Boc-L-His(π-Me)-D-Pro-L-(αMe)Val-L-Phe-OMe (3): yield 47% [after purification by flash chromatography (9:1, CH_2Cl_2−EtOH) followed by reversed-phase (prepacked C_{18} cartridge) chromatography (CH_3CN-H_2O gradient)]; mp 93−94 °C; $[\alpha]^{20}_D$ −34.0 (*c* 0.5, MeOH); R_f(I) 0.65, R_f(II) 0.60, R_f(III) 0.10; IR (KBr) ν_{max} 3350, 1743, 1684, 1639, 1516 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.49 (1H, s), 7.31−7.19 (6H, m), 7.07 (1H, d, *J* 8.7 Hz), 6.63 (1H, d, *J* 8.8 Hz), 6.42 (1H, s), 4.97 (1H, m), 4.53 (1H, m), 4.25 (1H, m), 3.96 (3H, s), 3.72 (3H, s), 3.61−3.49 (2H, m), 3.25−2.83 (4H, m), 2.11−1.87 (5H, m), 1.45 (3H, s), 1.41 (9H, s), 0.84 (3H, d, *J* 6.8 Hz), 0.73 (3H, d, *J* 6.8 Hz); MS (ESI-TOF) *m*/*z* calcd for $C_{33}H_{49}N_6O_7$ 641.3657, found 641.3705 $[M + H]^+$.

Cbz-Aib-L-(αMe)Phg-OMe: yield 81% [after purification by flash chromatography (99:1, CH_2Cl_2−EtOH)]; oil; $[\alpha]^{20}_D$ +29.8 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.95, R_f(III) 0.50; IR (film) ν_{max} 3312, 1729, 1676, 1514 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.53 (1H, s), 7.42−7.26 (10H, m), 5.35 (1H, s), 5.10 (2H, s), 3.68 (3H, s), 1.98 (1H, s), 1.53 (6H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{22}H_{27}N_2O_5$ 399.1914, found 399.1958 $[M + H]^+$.

Cbz-D-Pro-Aib-L-(αMe)Phg-OMe: yield 82% [after purification by flash chromatography (98:2, CH_2Cl_2-EtOH)]; mp 57−58 °C (EtOAc−PE); $[\alpha]^{20}_D$ +52.8 (*c* 0.5, MeOH); R_f(I) 0.95, R_f(II) 0.90, R_f(III) 0.35; IR (KBr) ν_{max} 3318, 1742, 1686, 1515 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.67 (1H, s), 7.44−7.25 (10H, m), 7.01 (1H, s), 5.05 (2H, s), 4.25 (1H, m), 4.25 (1H, m), 3.66 (3H, s), 3.59−3.36 (2H, m), 2.26−1.79 (4H, m), 1.97 (3H, s), 1.50 (3H, s), 1.46 (3H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{27}H_{34}N_3O_6$ 496.2442, found 496.2501 $[M + H]^+$.

Boc-L-His(π-Me)-D-Pro-Aib-L-(αMe)Phg-OMe (4): yield 39% [after purification by flash chromatography (94:6, CH_2Cl_2−EtOH) followed by reversed-phase (prepacked C_{18} cartridge) chromatography (CH_3CN-H_2O gradient)]; mp 103−104 °C; $[\alpha]^{20}_D$ +6.6 (*c* 0.5, MeOH); R_f(I) 0.35, R_f(II) 0.55, R_f(III) 0.05; IR (KBr) ν_{max} 3402, 3346, 1738, 1679, 1642, 1509 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.63 (1H, s), 7.48−7.24 (6H, m), 7.18 (1H, s), 7.06 (1H, s), 5.93 (1H, d, *J* 5.0 Hz), 4.47 (1H, m), 4.35 (1H, m), 3.76 (3H, s), 3.69 (3H, s), 3.60−3.38 (2H, m), 3.05−2.80 (2H, m), 2.08−1.87 (4H, m), 2.02 (3H, s), 1.57 (3H, s), 1.50 (3H, s), 1.42 (9H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{31}H_{45}N_6O_7$ 613.3344, found 613.3338 $[M + H]^+$.

Boc-L-His(π-Me)-L-(αMe)Val-NH *t*-Bu (5): yield 63% [after purification by flash chromatography (95:5, CH_2Cl_2−MeOH)]; mp 192−193 °C; $[\alpha]^{20}_D$ −23.6 (*c* 0.5, MeOH); R_f(I) 0.40, R_f(II) 0.35, R_f(III) 0.10; IR (KBr) ν_{max} 3337, 1703, 1675, 1523 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45 (1H, s), 6.89 (1H, s), 6.57 (1H, s), 6.32 (1H, s), 5.03 (1H, d, *J* 5.5 Hz), 4.15 (1H, m), 3.62 (3H, s), 3.11 (1H, dd, *J* 5.7, 16.0 Hz), 2.96 (1H, dd, *J* 7.8, 16.0 Hz), 2.14 (1H, m), 1.66 (3H, s), 1.44 (9H, s), 1.33 (9H, s), 0.88 (3H, d, *J* 4.4 Hz), 0.86 (3H, d, *J* 4.4 Hz); MS (ESI-TOF) *m*/*z* calcd for $C_{22}H_{40}N_5O_4$ 438.3075, found 438.3022 $[M + H]^+$.

Boc-L-His(π-Me)-D-(αMe)Val-NH *t*-Bu (6): yield 41% [after purification by flash chromatography (95:5, CH_2Cl_2−MeOH)]; mp 81−82 °C; $[\alpha]^{20}_D$ −2.0 (*c* 0.5, MeOH); R_f(I) 0.40, R_f(II) 0.35, R_f(III) 0.10; IR (KBr) ν_{max} 3355, 3314, 1686, 1511 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.49 (1H, s), 6.84 (1H, s), 6.63 (1H, s), 6.35 (1H, s), 5.27 (1H, d, *J* 5.1 Hz), 4.15 (1H, m), 3.59 (3H, s), 3.04 (2H, m), 2.25 (1H, m), 1.42 (9H, s), 1.38 (3H, s), 1.31 (9H, s), 0.84 (6H, m); MS (ESI-TOF) *m*/*z* calcd for $C_{22}H_{40}N_5O_4$ 438.3075, found 438.3046 $[M + H]^+$.

Boc-L-His(π-Me)-L-(αMe)Phe-NH *t*-Bu (7): yield 77% [after purification by flash chromatography (9:1, CH_2Cl_2−EtOH)]; mp 159−160 °C; $[\alpha]^{20}_D$ −55.8 (*c* 0.5, MeOH); R_f(I) 0.50, R_f(II) 0.60, R_f(III) 0.10; IR (KBr) ν_{max} 3415, 3283, 1691, 1657,

(17) (a) Toniolo, C.; Crisma, M.; Valle, G.; Bonora, G. M.; Polinelli, S.; Becker, E. L.; Freer, R. J.; Sudhanand; Balaji Rao, R.; Balaram, P.; Sukumar, M. *Pept. Res.* **1989**, *2*, 275−281. (b) Formaggio, F.; Pantano, M.; Crisma, M.; Toniolo, C.; Boesten, W. H. J.; Schoemaker, H. E.; Kamphuis, J.; Becker, E. L. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 953−956.

1508 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.43 (1H, s), 7.27−7.25 (3H, m), 7.10−7.07 (2H, m), 6.82 (1H, s), 6.79 (1H, s), 5.86 (1H, s), 4.99 (1H, d, *J* 3.9 Hz), 4.10 (1H, m), 3.59 (3H, s), 3.24 and 3.12 (2H, 2d, both *J* 13.6 Hz), 3.10 (1H, dd, *J* 6.0, 14.4 Hz), 2.91 (1H, dd, *J* 7.9, 14.4 Hz), 1.55 (3H, s), 1.38 (9H, s), 1.30 (9H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{26}H_{40}N_5O_4$ 486.3075, found 486.3109 $[M + H]^+$.

Boc-L-His(π-Me)-D-(αMe)Phe-NH *t*-Bu (8): yield 52% [after purification by flash chromatography (9:1, CH_2Cl_2−EtOH)]; oil; $[\alpha]^{20}_D$ −4.6 (*c* 0.5, MeOH); R_f(I) 0.50, R_f(II) 0.60, R_f(III) 0.10; IR (KBr) ν_{max} 3332, 3315, 1680, 1506 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.38 (1H, s), 7.25−7.23 (3H, m), 7.10−7.07 (2H, m), 6.78 (1H, s), 6.63 (1H, s), 6.06 (1H, s), 5.10 (1H, br s), 4.09 (1H, m), 3.58 (3H, s), 3.35 (1H, d, *J* 13.7 Hz), 3.08 (1H, d, *J* 13.7 Hz), 3.06 (1H, dd, *J* 6.0, 14.4 Hz), 2.84 (1H, dd, *J* 7.9, 14.4 Hz), 1.47 (3H, s), 1.37 (9H, s), 1.29 (9H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{26}H_{40}N_5O_4$ 486.3075, found 486.3072 $[M + H]^+$.

Boc-L-His(π-Me)-L-(αMe)Phg-NH *t*-Bu (9): yield 64% [after purification by flash chromatography (9:1, CH_2Cl_2−EtOH)]; oil; $[\alpha]^{20}_D$ −5.2 (*c* 0.5, MeOH); R_f(I) 0.55, R_f(II) 0.60, R_f(III) 0.10; IR (KBr) ν_{max} 3415, 3357, 1677, 1507 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.88 (1H, s), 7.36−7.27 (7H, m), 6.83 (1H, s), 5.17 (1H, s), 4.30 (1H, m), 3.44 (3H, s), 2.99 (2H, d, *J* 6.5 Hz), 1.86 (3H, s), 1.44 (9H, s), 1.20 (9H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{25}H_{38}N_5O_4$ 472.2918, found 472.2967 $[M + H]^+$.

Boc-L-His(π-Me)-D-(αMe)Phg-NH *t*-Bu (10): yield 56% [after purification by flash chromatography (9:1, CH_2Cl_2−EtOH)]; oil; $[\alpha]^{20}_D$ −26.6 (*c* 0.5, MeOH); R_f(I) 0.55, R_f(II) 0.60, R_f(III) 0.10; IR (KBr) ν_{max} 3416, 3358, 1685, 1680, 1506 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.80 (1H, s), 7.43 (1H, s), 7.36−7.24 (5H, m), 6.78 (1H, s), 5.20 (1H, s), 5.15 (1H, d, *J* 8.4 Hz), 4.25 (1H, m), 3.46 (3H, s), 2.96 (2H, m), 1.86 (3H, s), 1.44 (9H, s), 1.20 (9H, s); MS (ESI-TOF) *m*/*z* calcd for $C_{25}H_{38}N_5O_4$ 472.2918, found 472.2971 $[M + H]^+$.

Standard Conditions for Resolution of the Racemic Alcohol Substrate. In a thermostated reaction vessel (±)-*trans*-2-acetamidocyclohexanol (5.0 mg, 0.03 mmol) was dissolved in 3.1 mL of toluene (distilled from Na) by heating at 50 °C. When all reagent was dissolved, 100 μL of a 16.3 nM stock solution of catalyst (0.0016 mmol of peptide) were added at 25 °C. After 20 min, acetic anhydride (25 μL, 0.265 mmol, 8.8 equiv) was introduced. The reaction mixture was allowed to stir at 25 °C. Aliquots of 50 μL were removed, quenched with methanol, and directly tested by GC analysis as described below.

Assay of Conversion. The extent of reagent conversion was determined using a Hewlett-Packard model 5890 gas -chromatograph equipped with a 15 m wide bore ($Ø_{ID}$ = 0.53 mm) AT-1701 column (Alltech). Conditions: isotherm temperature, 150 °C; flow rate, 1 mL/min. Retention times: 15 min (products), 18 min (reagents). Conversions were determined in analogy to the method reported by Miller and co-workers.[2a,d]

Assay of Enantiomeric Purity. Enantiomers of starting material and product were separated by chiral GC employing a Shimadzu model GC-14B gas chromatograph equipped with a 30 m Chiraldex G-TA capillary column (Alltech). Conditions: flow rate, 120 kPA; initial temperature, 128 °C (0−26 min); temperature ramp, 1 °C/min; final temperature, 135 °C. Reaction times: 22 min [(*R*,*R*) product], 24 min [(*S*,*S*) product], 41 min [(*R*,*R*) reagent], 45 min [(*S*,*S*) reagent]. k_{rel} = *s* (selectivity) values were calculated according to the method of Kagan.[12]

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Supporting Information Available: ^1H NMR spectra of all synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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