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Synthesis of Protected 2-Pyrrolylalanine for Peptide Chemistry and Examination of Its Influence on Prolyl Amide Isomer Equilibrium

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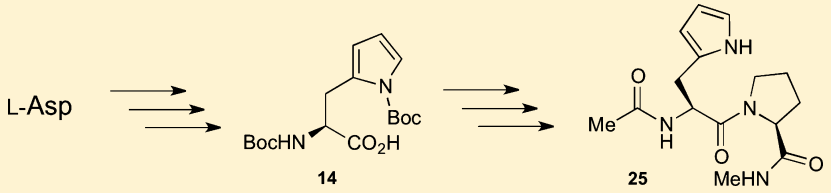
Supporting Information



ABSTRACT: Protected enantiopure 2-pyrrolylalanine was synthesized for application in peptide science as an electron-rich arylalanine (histidine) analog with π-donor capability. (2*S*)-*N*-(Boc)-*N*′-(Phenylsulfonyl)-, (2*S*)-*N*,*N*′-bis-(phenylsulfonyl)-, and (2*S*)-*N*,*N*′-bis-(Boc)-3-(2-pyrrolyl)alanines (**10**, **3**, and **14**, respectively) were made in 13−17% overall yields and six to seven steps from oxazolidine β-methyl ester **4**. Homoallylic ketone **5** was prepared by a copper-catalyzed cascade addition of vinylmagnesium bromide to ester **4** and converted to pyrrolyl amino alcohol **7** by olefin oxidation and Paal-Knorr condensation. Protecting group shuffle and oxidation of the primary alcohol enabled the synthesis of pyrrolylalanines. The bis-Boc analog **14** proved useful in peptide chemistry and was employed to make *N*-acetyl-pyrrolylalaninyl-proline *N*′′-methylamide **25**. A study of the influence of the pyrrole moiety on the prolyl amide isomer equilibrium of **25** using ^{1}H NMR spectroscopy in chloroform, DMSO, and water demonstrated that the pyrrolylalanine peptide exhibited behavior and conformations different from those of other arylalanine analogs.

INTRODUCTION

Structurally diverse amino acid derivatives have widespread use in the physical and life sciences.[1] For example, phenylalanine[2a−f] and histidine[2f−h] have been used to study the importance of π-interactions for peptide folding, recognition, and biological activity[4−8] because they possess differing degrees of aromatic-ring electron density.[2,3] At the *N*-terminus of proline in peptides, Phe may augment the prolyl amide *cis*-isomer population, decrease the rate of amide isomerization, and limit protein folding.[8−14] Stabilization of the *cis*-isomer has been suggested to be due to an electrostatic interaction between the prolyl amide nitrogen and the Phe aromatic π-system,[5a,f,9] in part because the prolyl amide isomer population has been modulated by varying the electronic density of the side chain of the residue *N*-terminal to proline in peptides.[15−17] For example, in Ac-Xaa-Pro-NHMe, the *cis*-amide isomer population was reduced on changing the Xaa residue from Phe to electron deficient arylalanine residues,[17] such as pyradizinylalanine.[16]

Histidine analogs (i.e., thienylalanine, furylalanine, triazolylalanine, tetrazolylalanine, thiazolylalanine, imidazolylalanine, and pyrazolylalanine) have been used to study the importance of the hydrogen bond acceptor and donator properties of the imidazole side chain.[2f−h] Components of naturally occurring peptides from marine organisms,[18] amino acids bearing five-membered heterocycle side chains have been employed in peptidomimetics,[19] macromolecular scafolds,[20] and high-throughput synthesis.[21] Although pyrrolylalanine (**1**,[22c] Figure 1) represents a promising histidine surrogate, few syntheses give an enantiopure product suitable for peptide chemistry.[22,23]

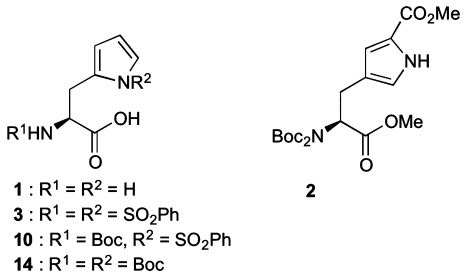


Figure 1. Protected pyrrolylalanine analogs.

For example, the natural pyrrolylalanine analog from the poisonous mushroom *Clitocybe acromelalga*, (2*S*)-3-(2-carboxy-4-pyrrolyl)alanine (**2**), has been synthesized in protected form as a racemate[22f] and in optically active form from L-aspartic acid.[23a−c] Enantiomerically pure (>94% ee) (2*S*)-*N*-Cbz-3-(2-pyrrolyl)alanine derivatives were synthesized by enantioselective hydrogenation of the corresponding methyl and *tert*-butyl (*Z*)-α,β-didehydroalaninates.[23d] (2*S*)-*N*-(Boc)-*N*′-Acetyl-3-(2-pyrrolyl)alanine was also synthesized from L-aspartic acid by a route featuring ring-closing metathesis and aromatization to form the heterocycle.[23e] Prior to our preliminary research,[17] to

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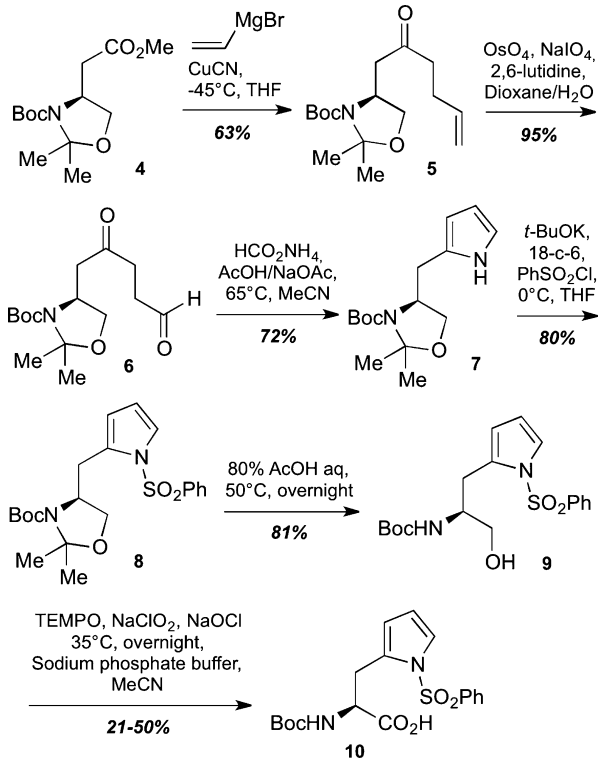
the best of our knowledge,[22,23] pyrrolylalanine has been used only once in the synthesis of a peptide analog. Racemic *N*-Cbz-*N*′-(Boc)-3-(2-pyrrolyl)alanine was introduced into a thyrotropin-releasing hormone (TRH, pGlu-His-Pro-NHMe) analog to investigate the role of the histidine residue; however, no biological activity was reported.[22b]

In the context of research on biologically active peptides bearing histidine residues,[24,25] we have pursued the development of an effective methodology for the synthesis of enantiomerically pure 3-(2-pyrrolyl)alanine in a form suitable for introduction into peptides. Recently, we communicated the synthesis of *N*,*N*′-bis-(phenylsulfonyl)-3-(2-pyrrolyl)alanine (**3**, Figure 1) by a route featuring a copper-catalyzed cascade addition of vinylmagnesium bromide to aspartic acid-derived *β*-amino ester **4** (Scheme 1).[17] Although pyrrolylalanine was

Scheme 1. Synthesis of *N*-(Boc)-*N*′-(Phenylsulfonyl)-3-(2-pyrrolyl)alanine 10



obtained in 13% overall yield from **4** and introduced into a dipeptide, attempts to remove the sulfonyl groups were unsuccessful. Alternative strategies have now been developed for the synthesis of enantiopure (2*S*)-3-(2-pyrrolyl)alanine derivatives, which are more suitable for peptide synthesis as validated by incorporation into Ac-Xaa-Pro-NHMe dipeptide models. The influence of (2*S*)-2-pyrrolylalanine on the prolyl amide isomer population was subsequently examined by comparing measurements of *cis*-isomer populations with related arylalanine derivatives.

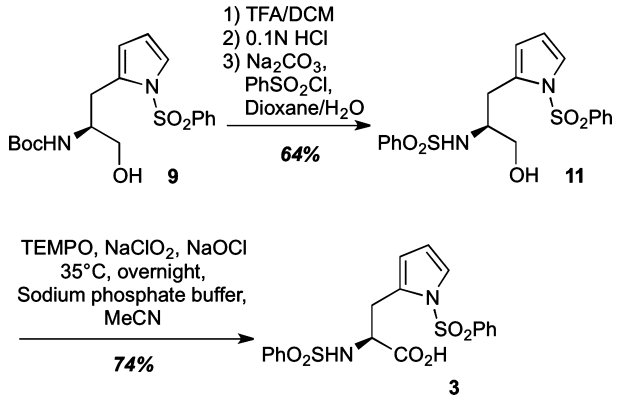
RESULTS AND DISCUSSION

Protected 2-Pyrrolylalanine Synthesis. Pyrrole **7** was prepared in three steps from *β*-amino ester **4**, which was obtained in four steps with a 47% yield from L-aspartic acid as the chiral educt.[16] The Cu-catalyzed cascade addition of excess vinylmagnesium bromide to methyl ester **4** in THF at −45 °C gave homoallylic ketone **5** in 63% yield (Scheme 1).[16,26] Pyrrole **7** was prepared by olefin oxidation and Paal−Knorr condensation. *γ*-Ketoaldehyde **6** was made from olefin **5** using a mixture of OsO_4−$NaIO_4$ and 2,6-lutidine in dioxane/water in 95% yield.[27] Condensation of *γ*-ketoaldehyde **6** with ammonium formate in the presence of a mixture of NaOAc/AcOH (1:1, 1 eq w/w) in acetonitrile at 65 °C gave pyrrole **7** in 72% yield.

In earlier research on bipyrrole and prodigiosin analogs,[28] sulfonamide protection of the ring nitrogen prevented destruction of the electron-rich pyrrole during double-bond oxidation.[29] To oxidize the primary alcohol to the carboxylic acid without pyrrole degradation,[30] pyrrole **7** was thus protected as the phenylsulfonyl counterpart **8** with *t*-BuOK, 18-c-6, and phenylsulfonyl chloride in THF in 80% yield. Subsequently, oxazolidine **8** was ring opened using 80% aqueous acetic acid at 50 °C overnight to produce *N*-(Boc)amino alcohol **9** in 81% yield.

Oxidation of *N*-(Boc)amino alcohol **9** to (2*S*)-*N*-(Boc)-*N*′-(phenylsulfonyl)-3-(2-pyrrolyl)alanine **10** proved challenging. Poor yields of acid **10**, at best 21−50%, from oxidation of **9** using the TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) free radical, sodium chlorite, and sodium hypochlorite in a sodium phosphate-buffered acetonitrile solution[16,31,32] inspired a change in amine protection from Boc to benzenesulfonyl. The carbamate was removed using 25% trifluoroacetic acid (TFA) in dichloromethane, and the sulfonamide was installed using phenylsulfonyl chloride and Na_2CO_3 in dioxane/water to provide (2*S*)-*N*,*N*′-bis-(phenylsulfonyl)-3-(2-pyrrolyl)alaninol **11** in 64% overall yield (Scheme 2). Using the TEMPO

Scheme 2. Synthesis of *N*,*N*′-Bis-(phenylsulfonyl)-3-(2-pyrrolyl)alanine 3



oxidation conditions described above, (2*S*)-*N*,*N*′-bis-(phenylsulfonyl)-3-(2-pyrrolyl)alanine **3** was obtained from *N*-(phenylsulfonyl)amino alcohol **11** in 74% yield. Although the 2-pyrrolylalanine targets were achieved because of sulfonamide protection, low yields were obtained in attempts to remove the sulfonyl group (i.e., TBAF in THF, SmI_2 in DMPU/THF, and Mg in MeOH), which plagued efforts to employ amino acids **3** and **10** in peptide synthesis.[17]

To facilitate protecting group removal during peptide synthesis, *N*,*N*′-bis-(Boc)-3-(2-pyrrolyl)alanine **14** was subsequently targeted. Pyrrole **7** was protected with di-*tert*-butyl dicarbonate and catalytic 4-(dimethylamino)pyridine (DMAP) in acetonitrile to produce *N*-(Boc)pyrrole **12** in 98% yield (Scheme 3).[33] Removal of the oxazolidine from **12** with 80% aqueous acetic acid gave *N*,*N*′-bis-(Boc)-3-(2-pyrrolyl)alaninol

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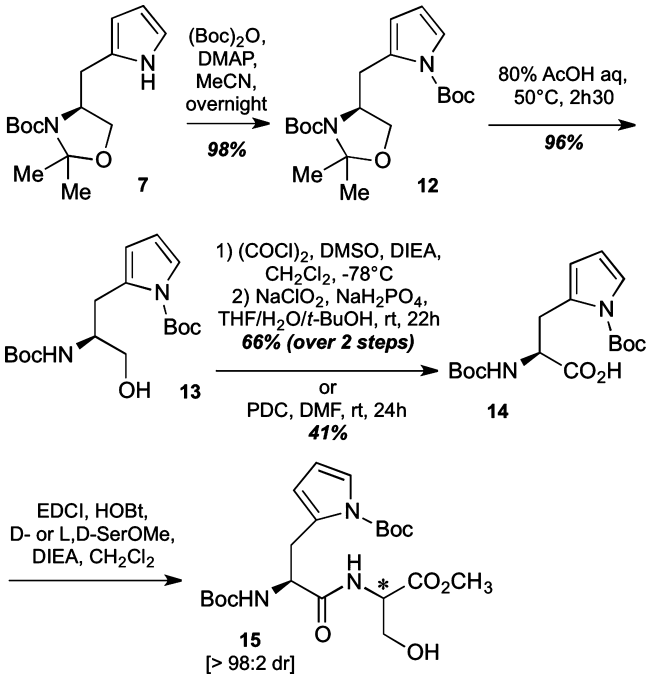
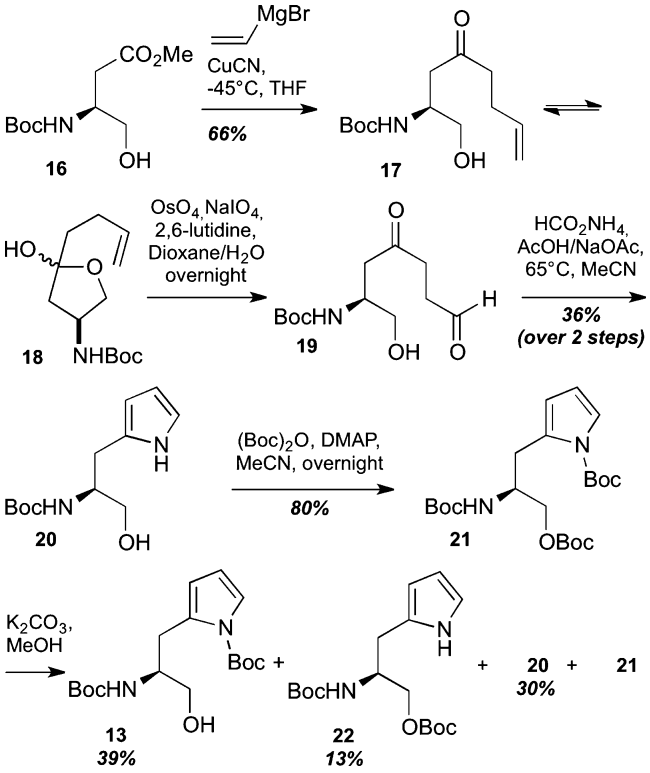
Scheme 3. Synthesis of *N,N′*-Bis-(Boc)-3-(2-pyrrolyl)alanine 14



Scheme 4. Synthesis of *N,N′*-Bis-(Boc)-3-(2-pyrrolyl)alaninol 13 from Methyl Ester 16



13 in 96% yield. Most attempts (e.g., TEMPO,[32] $NaIO_4$–$RuCl_3$,[34] H_5IO_6–CrO_3,[35] Pt/O_2[36]) failed, however, to convert alcohol **13** to carboxylic acid **14**, except for pyridinium dichromate (PDC) in DMF,[37] which produced *N,N′*-bis-(Boc)-3-(2-pyrrolyl)alanine in 41% yield. Alternatively, acid **14** was produced by a two-step sequence featuring oxidation to the aldehyde with oxalyl chloride and DMSO,[38] followed by $NaClO_2$ oxidation in 66% overall yield;[39] however, the specific rotation of amino acid **14** was lower [−12.61 vs −19.14 (*c* 9.6 × 10^{-3}, $CHCl_3$)] after the two-step process, suggesting that the amino aldehyde intermediate was configurationally labile.[40]

The enantiomeric purity of *N,N′*-bis-(Boc)-3-(2-pyrrolyl)-alanine **14** was ascertained after conversion to the diastereomeric dipeptides **15** by coupling to L- and D,L-serine methyl ester hydrochloride using *N*-ethyl *N′*-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDCI), 1-hydroxybenzo-triazole (HOBt), and DIEA in dichloromethane. Observation of the crude dipeptides **15** and measurement of the diastereomeric *α*-proton multiplets of the 3-pyrrolylalanine residue at 4.74 and 4.84 ppm in the ^{1}H NMR spectra in C_6D_6, during incremental additions of (*S,R*)-**15** in a sample of (*S,S*)-**15**, demonstrated (*S,S*)-*N,N′*-bis-(Boc)-3-(2-pyrrolyl)alaninyl-serine methyl ester to have a >98:2 diastereomeric ratio. Hence, (2*S*)-3-(2-pyrrolyl)alanine **14** is considered to be of the same high enantiomeric purity.

In an alternative route, *N,N′*-bis-(Boc)-3-(2-pyrrolyl)alanine **14** was obtained from alcohol **16** (Scheme 4). Reduction of the *α*-carboxylate of *β*-methyl *N*-(Boc)aspartate gave alcohol **16**.[41] Homoallylic ketone **17** was obtained in 66% yield on treatment of **16** with vinylmagnesium bromide and catalytic CuCN, followed by purification by flash chromatography.[16] Homoallylic ketone **17** (R_f = 0.36, 50:50 ethyl acetate/hexane) existed in equilibrium with hemiacetal **18** (R_f = 0.77, 50:50 ethyl acetate/hexane). Oxidation of the mixture of **17** and **18** with $NaIO_4$, followed by condensation of the resulting *γ*-ketoaldehyde **19** with ammonium formate, delivered pyrrole **20** in 36% yield over two steps. Although selective pyrrole nitrogen protection of *N*-(Boc)-3-(2-pyrrolyl)alaninol **20** was unsuccessful, *N,N′,O*-tris-(Boc)-3-(2-pyrrolyl)alaninol **21** was prepared in 80% yield by employing excess $(Boc)_2O$ and DMAP in acetonitrile. In an attempt to selectively cleave the *O*-Boc group, treatment of **21** with K_2CO_3 in MeOH yielded a separable mixture of *N,N′*-bis-(Boc)-3-(2-pyrrolyl)alaninol **13** (35–39% yield), *N,O*-bis-(Boc)-3-(2-pyrrolyl)alaninol **22**, *N*-(Boc)-3-(2-pyrrolyl)alaninol **20**, and starting material **21**, contingent on reaction time. Recycling compounds **20**–**22** through a protection and deprotection process augmented the yield of *N,N′*-bis-(Boc)-3-(2-pyrrolyl)alaninol **13**.

Synthesis and Conformational Analysis of Ac-Xaa-Pro-NHMe Dipeptide Models. Steric effects between amino acid side chains usually favor the *trans*- over the *cis*-amide isomer. In the case of amides *N*-terminal to proline, however, the tertiary amide may adopt significant amounts of the *cis*-isomer, which are augmented by phenylalaninyl residues relative to less bulky alaninyl counterparts.[9] Stacking of the aromatic and pyrrolidine rings has been suggested to account for aromatic residues *N*-terminal to proline augmenting the prolyl *cis*-amide population relative to nonaromatic amino acids in dipeptide models, such as Ac-Xaa-Pro-NHMe and Ac-Xaa-5-*t*-BuPro-NHMe.[9] Via examination, the effect of aromatic ring electron density on the magnitude of the stacking interaction, the *π*-deficient arylalanine, (2*S*)-*N*-Boc-3-(6-methyl-pyridazinyl)alanine (Pal), was previously synthesized and incorporated into the dipeptide model, Ac-Xaa-Pro-NHMe, in which Pal significantly reduced the prolyl amide *cis*-isomer population relative to Phe.[16] To further explore the stacking interaction, the placement of the *π*-enriched arylalanine analog, (2*S*)-pyrrolylalanine *N*-terminal to proline, has now been examined with the expectation to augment the prolyl *cis*-amide

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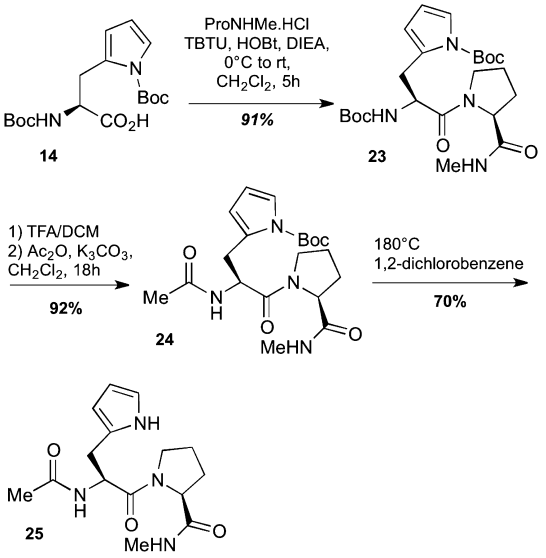
isomer population by increasing electron density of the π-system. Dipeptide models Ac-Xaa-Pro-NHMe **25**–**28** were synthesized to perform head-to-head comparisons between arylalanines with different π-density.

N,*N*′-bis-(Boc)-3-(2-pyrrolyl)alanine **14** was coupled to proline *N*″-methylamide hydrochloride using TBTU, HOBT, and DIEA to give *N*,*N*′-bis-phenylsulfonyl- and *N*,*N*′-bis-(Boc)-3-(2-pyrrolyl)alaninyl-proline *N*″-methylamides **23** in 91% yield (Scheme 5). Selective removal of the Boc group from

Scheme 5. Synthesis of *N*-(Acetyl)dipeptide *N*″-Methylamide 25

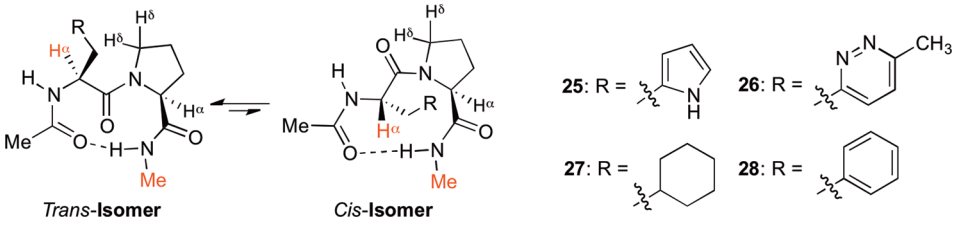


the α-amine without loss of the *N*-Boc-pyrrole was accomplished by treating dipeptide **23** with 25% TFA in dichloromethane. *N*-Acetyl-*N*′-(Boc)-3-(2-pyrrolyl)alaninyl-proline *N*″-methylamide **24** was produced by N-acetylation with acetic anhydride and potassium carbonate in dichloromethane in 92% overall yield from **23**. Removal of the Boc group from the pyrrole of **24** was accomplished by thermolysis with heating at 180 °C in 1,2-dichlorobenzene to give *N*-acetyl-3-(2-pyrrolyl)-alaninyl-proline *N*′′-methylamide **25** in 70% yield.[42] For comparisons, *N*-acetyl-pyridizinyl-, cyclohexylalaninyl, and phenylalaninyl-proline *N*′′-methylamides **26**–**28** were prepared as previously described.[16]

The prolyl major *trans*- and minor *cis*-amide isomers were analyzed by NMR spectroscopy in chloroform on the basis of their characteristic nuclear Overhauser effects between the α-proton of the *N*-terminal Xaa residue and either the α-proton or the δ-protons of the proline residue in the NOESY spectra in chloroform.[43] The relative populations of the amide *cis*- and *trans*-isomers *N*-terminal to the prolyl residues of dipeptide models **25**–**28** were ascertained by ^{1}H NMR spectroscopy in chloroform, dimethyl sulfoxide, and water (Table 1). The percentages of amide *cis*- and *trans*-isomer populations of **25** were measured by integration of the isomeric signals for the 2-pyrrolylalanine α-proton in the ^{1}H NMR spectra in chloroform and dimethyl sulfoxide, in which the α-proton signal for the *trans*-isomer appeared downfield from that of the *cis*-isomer (Table 2). In water, isomer populations of **25** were measured by integration of the *N*′′-methyl doublets in the ^{1}H NMR spectra. On the other hand, the percentages of amide *cis*- and *trans*-isomer populations of **26** were measured by integration of the isomeric signals for the pyridazinylalanine α-protons in the ^{1}H NMR spectra of the three solvents. In the case of the cyclohexylalanine and phenylalanine analogs, **27** and **28**, respectively, isomer populations were measured by integration of the *N*′-methyl doublets in the ^{1}H NMR spectra of the three solvents. In addition, the *N*″-methylamide and acetamide proton signals of the major *trans*-amide conformers of **25**–**28** were assigned based on their characteristic coupling patterns as confirmed by the TOCSY spectra in chloroform, dimethyl sulfoxide, and water (Table 1).

To distinguish aromatic from hydrophobic interactions, phenylalanine was compared with cyclohexylalanine. The choice of solvent enhanced or mitigated the effects of the side chain *N*-terminal to proline with the pyrrolidine ring. In the relatively nonpolar solvent chloroform, the Cha and Phe analogs **27** and **28**, respectively, exhibited essentially the same effect on the isomer equilibrium. On the other hand, in the relatively polar solvents, DMSO and water, Phe peptide **28** exhibited a significant increase in *cis*-isomer population relative to Cha peptide **27**, which experienced a decrease in *cis*-isomer population relative to those observed in chloroform. The aromatic effect is thus more pronounced in polar (DMSO and water) rather than nonpolar (chloroform) solvent. In addition, the placement of π-deficient pyridazinylalanine *N*-terminal to proline in **26** gave lower *cis*-isomer populations relative to phenylalanine peptide **28** in all three solvents suggesting that

Table 1. Influence of Solvent on Chemical Shifts and Amide Isomer Equilibrium of 25-28[a]



		% *cis*-isomer			(CDCl$_3$)[a]		(CDCl$_3$ → DMSO)[a]		(CDCl$_3$ → D$_2$O)[a]	
entry	N-terminal residue	D$_2$O	DMSO	CDCl$_3$	δ NH^{Xaa}	NH^{Me}	Δδ NH^{Xaa}	NH^{Me}	Δδ NH^{Xaa}	NH^{Me}
(*S*)-**25**	L-Pyr	27	25	0	6.39	5.93	1.79	1.53	1.72	1.41
(*S*)-**26**	L-Pal	23	12	0	6.50	7.80	1.82	−0.11	1.89	0.18
(*S*)-**27**	L-Cha	17	14	21	6.08	6.69	1.92	0.98	2.04	1.05
(*S*)-**28**	L-Phe	31	35	20	6.24	6.36	1.99	1.16	1.95	1.09

[a]Values are for the major conformer at 5 mM and 25 °C, determined by NMR spectroscopy at 700 and 500 MHz for **25** and **26**–**28**, respectively.

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Table 2. Chemical Shifts and Integrations of Peaks used in the Conformational Analysis of 25[a]

	δ (I)			
	H$^{\alpha}$(Pyr)		N′-Me	
solvent	*cis*-**25**	*trans*-**25**	*cis*-**25**	*trans*-**25**
$CDCl_3$	–	4.86 (m) (1.00)		
D_2O			2.55 (d) (1.10)	2.60 (d) (3.00)
DMSO	4.33 (q) (0.28)	4.60 (q) (0.86)		

[a]Chemical shifts are in parts per million, and integration values are given in parentheses.

the diminished π-electron donor prolyl electron acceptor interaction destabilized the *cis*-isomer.[16]

Although insertion of π-enriched pyrrolylalanine into peptide **25** was expected to strengthen the electrostatic interaction, the effects on *cis*-isomer population were modest at best. Relative to pyridazinylalanine peptide **26**, pyrrolylalanine peptide **25** increased *the cis*-isomer population by only 4% in water and 13% in dimethyl sulfoxide; however, no change was seen in chloroform by varying pyridazinylalanine to pyrrolylalanine, and only the *trans*-isomer was observed. Relative to phenylalanine peptide **28**, pyrrolylalanine peptide **25** exhibited significantly lower *cis*-isomer populations in all three solvents. Evidently, the aromatic ring of pyrrolylalanine had a limited effect on the π-electron donor prolyl electron acceptor interaction, and other factors appear to play greater roles in controlling the isomer equilibrium, such as potential hydrogen bonding to the proton of the pyrrole ring as well as nitrogen−nitrogen lone-pair repulsion.

To try and gain a better understanding of the deviant behavior of the pyrrolylalanine peptide **25**, the chemical shifts of the amide protons of the major conformers of peptides **25**−**28** were compared in the different solvent systems. The methylamide proton typically came downfield of the acetamide proton in peptides **26**−**28** in chloroform; however, in the spectrum of pyrrolylalanine peptide **25**, the methylamide proton (5.93 ppm) was upfield relative to the signal for the acetamide proton (6.39 ppm). To distinguish the relative exposures of the amide protons to the solvent, the change in chemical shift of their signals was examined upon switching to a more polar solvent because in studies of cyclic[44] and model linear[45] peptides, polar solvents, which may form hydrogen bonds, cause the signals of exposed amide protons to downfield shift yet have limited influence on the chemical displacement of solvent-shielded protons. Downfield shifts of proton signals upon changes in solvent from chloroform to DMSO and water were significantly less for the methylamide ($\Delta\delta$ = 0.11−1.16 ppm) relative to the acetamide ($\Delta\delta$ = 1.82−2.04 ppm) for the *trans*-amide conformers of **26**−**28**. For pyrrolylalanine peptide **25**, in contrast, when the solvent was switched from chloroform to dimethyl sulfoxide and water the downfield shifts for the proton signals were slightly less for the methylamide ($\Delta\delta$ = 1.53 and 1.41 ppm) than the acetamide ($\Delta\delta$ = 1.79 and 1.72 ppm). The effects of the solvent on the amide proton chemical shift were in accord with the *trans*-amide conformer of peptides **26**−**28**, adopting a preferred β-turn geometry in which the methylamide is solvent-shielded in a hydrogen bond with the acetamide carbonyl. On the other hand, the solvent effects on the amide proton signals of **25** were inconsistent with those of peptides **26**−**28**. From the examination of the behavior of the amide proton signals relative to the conformers of peptides **26**−**28**, the conformational preferences of peptide **25** were different, evidently as a result of the unique interactions of the pyrrole ring with the other functionality in the peptide chain.

CONCLUSION

Effective methods for synthesizing enantiopure protected 3-(2-pyrrolyl)alanine analogs have been developed starting from aspartic acid. (2*S*)-*N*,*N*′-Bis-(Boc)-3-(2-pyrrolyl)alanine **14** (>96% ee) was synthesized in 17% overall yield in six steps from oxazolidine β-methyl ester **4** and inserted successfully into dipeptide model Ac-Xaa-Pro-NHMe **25**. Conformational analysis of **25** and comparisons with related peptides **26**−**28** demonstrated that the pyrrole side chain had limited influence on the prolyl *cis*-amide isomer populations compared to those of other aromatic residues, such as phenylalanine in peptide **28**, and it adopted different conformational preferences. 3-(2-Pyrrolyl)alanine **14** can be introduced into peptides effectively and may be employed as an asparagine or histidine surrogate. Future applications of **14** that involve the study of the structure−function relationships in peptide science and medicinal chemistry are under investigation and will be reported in due time.

EXPERIMENTAL SECTION

Materials and Methods. Unless otherwise noted, all reactions were run under a nitrogen atmosphere and distilled solvents were transferred by syringe. Anhydrous solvents (THF, CH_3CN, DMF, and CH_2Cl_2) were obtained by passage through solvent-filtration systems (GlassContour, Irvine, CA). DIEA was distilled over ninhydrin and CaH_2. Final reaction mixture solutions were dried over $MgSO_4$ or Na_2SO_4. Chromatography was conducted on 230−400 mesh silica gel, and TLC was on glass-backed silica plates. All compounds were purified by flash column chromatography on silica gel.[46] L-Serine methylester hydrochloride and D,L-serine methyl ester hydrochlorides were purchased from Aldrich, and proline *N*-methylamide hydrochloride was synthesized from *N*-Boc-proline.[9] ^{1}H NMR spectra were measured in $CDCl_3$ and C_6D_6 at 700/400/300 MHz and referenced to $CDCl_3$ (7.26 ppm) and C_6D_6 (7.16 ppm). For compound **26**, ^{1}H NMR spectra were also measured in $CDCl_3$, DMSO-d_6, and D_2O at 700 MHz. ^{13}C NMR spectra were measured in $CDCl_3$ at 175/100/75 MHz and referenced to $CDCl_3$ (77.16 ppm). The chemical shifts for the carbons and the protons of the minor isomers are reported in parentheses and brackets, respectively. Mixing times of 800 and 80 ms were used for the NOESY and TOCSY spectra, respectively. Relative populations of prolyl *cis*- and *trans*-amide isomers of compounds **23**−**25** were determined in choroform at 5 mM at 25 °C. HRMS measurements were made on a LC-MSD TOF (Agilent) mass analyzer.

(*S*)-4-(1-*tert*-Butyloxycarbonyl-1*H*-pyrrol-2-ylmethyl)-2,2-dimethyl-oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (12). A stirred solution of pyrrole **7** (prepared according to ref 17, 2.71 g, 9.66 mmol) in MeCN (140 mL) at room temperature was treated with DMAP (120 mg, 0.98 mmol) and $(Boc)_2O$ (2.57 g, 11.77 mmol) and stirred overnight. The volatiles were removed by evaporation, and the residue was partitioned between H_2O (300 mL) and Et_2O (300 mL). The layers were separated and the aqueous phase was extracted with

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Et_2O (2 × 300 mL). The combined organic extracts were washed with H_2O (300 mL) and brine (300 mL), dried (Na_2SO_4), filtered, and evaporated to give *N*-(Boc)pyrrole **12** (3.58 g, 98%) as a sticky yellow oil: TLC R_f 0.37 (10:90 EtOAc/hexane); $[\alpha]_D^{20}$ −17.91 (*c* 8 × 10^{-3}, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) indicated a 1:1 mixture of carbamate isomers δ 1.35 (s, 12H), 1.41−1.55 (2 s partially overlapped, 14H), 1.59 (s, 18H), [1.68 (s, 4H)], 3.09−3.30 (2 m partially overlapped, 4H), 3.81 (d, *J* = 8.8 Hz, 2H), 3.94 (m, 2H), 4.25−4.39 (2 m partially overlapped, 2H), 5.85−5.97 (2 br s partially overlapped, 2H), 6.02−6.12 (2 br s partially overlapped, 2H), 7.19 (s 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.3, 132.6, 121.2, 113.3 (112.1), 110.5 (110.2), 94.2 (93.5), 83.5, 79.6, 67.7 (67.2), 56.7, 33.3 (32.1), (28.6) 28.5, 28.3, (27.9) 27.5, (24.8) 23.5; HRMS (ESI) calcd *m*/*z* for $C_{20}H_{32}N_2NaO_5$ [(M + Na)$^+$] 403.2203, found *m*/*z* 403.2221.

(2*S*)-*N*,*N*′-Bis-(Boc)-3-(2-pyrrolyl)alaninol (13). Oxazolidine **12** (3.48 g, 9.17 mmol) was dissolved in 180 mL of 80% aq AcOH (5.6 mL per 1 mmol of oxazolidine). The mixture was stirred at 50 °C and monitored by TLC [**12**: R_f 0.95 (15:85 EtOAc/dichloromethane); **13**: R_f 0.77 (15:85 EtOAc/dichloromethane)]. After 2.5 h, the reaction mixture was cooled to room temperature, concentrated by rotary evaporation, and treated with brine (200 mL). The aqueous layer was extracted with EtOAc (3 × 300 mL). The combined organic phases were washed with H_2O (200 mL), $NaHCO_3$ (100 mL), and brine (200 mL), dried ($MgSO_4$), filtered, and evaporated to a residue, which was purified by silica gel column chromatography (2% methanol in dichloromethane) to give alcohol **13** as a pale yellow viscous oil after lyophilization (2.99 g, 96%): TLC R_f 0.22 (3:97 MeOH/dichloromethane); $[\alpha]_D^{20}$ −21.16 (*c* 7.6 × 10^{-3}, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.6 (s, 9H), 2.93−3.10 (br m, 2H), 3.19 (dd, *J* = 6.4, 14.8 Hz, 1H), 3.58 (dd, *J* = 3.6, 10.8 Hz, 1H), 3.65 (br d, *J* = 10.8 Hz, 1H), 3.86 (m, 1H), 6.06−6.12 (t, *J* = 3.4 Hz and br s partially overlapped, 2H), 7.17 (dd, *J* = 2, 3.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 150.3, 132.0, 121.6, 114.0, 110.6, 84.3, 79.6, 65.1, 53.7, 29.9, 28.6, 27.9; HRMS (ESI) calcd *m*/*z* for $C_{17}H_{28}N_2NaO_5$ [(M + Na)$^+$] 363.1890, found *m*/*z* 363.1905.

(2*S*)-*N*,*N*′-Bis-(Boc)-3-(2-pyrrolyl)alanine (14) from PDC Oxidation. A stirred solution of alcohol **13** (56.5 mg, 0.166 mmol) in DMF (0.6 mL) at room temperature was treated with PDC (312 mg, 0.830 mmol), stirred for 24 h, and partitioned between H_2O (5 mL) and ethyl acetate (5 mL). The mixture was extracted with ethyl acetate (3 × 3 mL). The combined organic phases were washed with H_2O (5 mL) and brine (5 mL), dried ($MgSO_4$), filtered, and evaporated to give a residue, which was purified by silica gel column chromatography (2−5% methanol in dichloromethane) to give acid **14** as a white foam (24.2 mg, 41%): TLC R_f 0.22 (3:97 MeOH/dichloromethane); $[\alpha]_D^{20}$ −21.16 (*c* 7.6 × 10^{-3}, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20−1.50 (2 s, 9H), 1.61 (s, 9H), 3.20−3.33 (m, 1H), 3.50−3.68 (m, 1H), 4.55 (br s, 1H), 5.36 (br d, *J* = 6.4 Hz, 1H), 6.09 (d, *J* = 2.8 Hz, 1H), 7.22 (s, 1H), 8.66 (br s, 0.4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 150.0, 148.4, 130.3, 122.2, 114.4, 110.5, 84.4, 80.4, 54.2, 30.9, 28.5, 28.2; HRMS (ESI) calcd *m*/*z* for $C_{17}H_{26}N_2NaO_6$ [(M + Na)$^+$] 377.1683, found *m*/*z* 377.1702.

(2*S*)-*N*,*N*′-Bis-(Boc)-3-(2-pyrrolyl)alanine (14) by Way of Aldehyde Intermediate. A solution of oxalyl chloride (50 μL, 0.565 mmol) in $CHCl_2$ (1 mL) at −78 °C was slowly treated with a solution of DMSO (48 μL, 0.678 mmol) in $CHCl_2$ (1 mL), stirred for 10 min, treated with a solution of alcohol **13** (38.5 mg, 0.113 mmol) in $CHCl_2$ (2 mL), stirred for 30 min, treated with DIEA (197 μL, 113 mmol), allowed to warm to 0 °C and stirred for 10 min, and quenched with brine (4 mL). The reaction mixture was extracted with $CHCl_2$ (3 × 10 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Without further purification, the aldehyde intermediate was used immediately in the next step.

The aldehyde in *t*-BuOH (3 mL) at room temperature was treated with 2-methyl-2-butene (96 μL, 0.904 mmol) in THF (9 mL), followed by dropwise addition of a solution of sodium chlorite (34 mg, 0.339 mmol) and potassium dihydrogenphosphate (42 mg, 0.339 mmol) in H_2O (9 mL). The mixture was stirred vigorously for 22 h at room temperature, partitioned between saturated aqueous NH_4Cl (45 mL) and ethyl acetate (45 mL), and the aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic phases were washed with brine (30 mL), dried ($MgSO_4$), filtered, and concentrated to give a residue, which was purified by column chromatography (1−5% MeOH in dichoromethane) to provide 2-pyrrolylalanine **14** (26.6 mg, 66%) as a white foam exhibiting identical spectroscopic characteristics as described above: $[\alpha]_D^{20}$ −12.61 (*c* 9.6 × 10^{-3}, $CHCl_3$).

(*S*,*S*)- and (*S*,*RS*)-*N*,*N*′-Bis-(Boc)-3-(2-pyrrolyl)alaninyl-serine Methyl Ester (15). A solution of 2-pyrrolylalanine **14** (30 mg, 0.085 mmol), HOBt (11.5 mg, 0.085 mmol), DIEA (44.5 μL, 0.085 mmol), and L-serine ester methyl hydrochloride (26.5 mg, 0.170 mmol) in CH_2Cl_2 (3.5 mL) was stirred at 0 °C for 15 min, treated with EDCI (13.3 mg, 0.085 mmol), and allowed to warm to room temperature. After the mixture had been stirred for 2.5 h, TLC showed that acid **14** [R_f 0.23 (5:95 EtOAc/dichloromethane)] was completely consumed and a new, less polar product, (*S*,*S*)-dipeptide **15**, was formed [R_f 0.29 (5:95 EtOAc/dichloromethane)]. The volatiles were evaporated under vacuum, and the residue was dissolved in EtOAc (15 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 5 mL), H_2O (5 mL), 1 N HCl (2 × 5 mL), H_2O (5 mL), and brine (5 mL), dried ($MgSO_4$), filtered, and evaporated to give a residue that was examined by 400 MHz ^{1}H NMR spectroscopy in C_6D_6. (*S*,*S*)-*N*,*N*′-Bis-(Boc)-3-(2-pyrrolyl)-alaninyl-serine methyl ester (*S*,*S*)-**15**: ^{1}H NMR (400 MHz, C_6D_6) δ 1.30−1.35 (2 s partially overlapped, 18H), 2.93 (br s, 1H), 3.14 (dd, *J* = 10.8, 14 Hz, 1H), 3.23 (s, 3H), 3.70 (dd, *J* = 2.6, 14.6 Hz, 1H), 3.82 (dd, *J* = 2.2, 11.2 Hz, 1H), 3.95 (br d, *J* = 3.95 Hz, 1H), 4.64 (m, 1H), 4.74 (m, 1H), 5.46 (br d, *J* = 7.2 Hz, 1H), 5.98 (2 s partially overlapped, 2H), 7.21 (br t, *J* = 2.2 Hz, 1H), 7.32 (br d, *J* = 6.8 Hz, 1H).

(*S*,*RS*)-*N*,*N*′-Bis-(Boc)-3-(2-pyrrolyl)alaninyl-serine methyl ester (*S*,*RS*)-**15** was prepared employing the same protocol except for the use of D,L-serine methyl ester: ^{1}H NMR (400 MHz, C_6D_6) δ 1.35 (s, 18H), 1.37 (s, 18H), 2.49 (br s, 1H), 2.67 (br s, 1H), 3.06−3.20 (m, 2H), 3.25 (s, 6H), 3.63−3.76 (m, 2H), 3.76−3.86 (m, 2H), 3.88−4.01 (m, 2H), 4.60 (m, 1H), 4.64 (m, 1H), 4.74 (m, 1H), 4.84 (m, 1H), 5.37 (br d, *J* = 6.4 Hz, 1H), 5.44 (br d, *J* = 6.4 Hz, 1H), 5.95−6.03 (m, 4H), 7.25 (s, 4H).

The measurement of the limits of detection of (*S*,*R*)-**15** in a sample of (*S*,*S*)-**15** was performed by integration of the signals of the diastereomeric α-proton multiplets at 4.74 and 4.84 ppm in ^{1}H NMR spectra in C_6D_6 during incremental additions of (*S*,*RS*)-**15**, which demonstrated that (*S*,*S*)-*N*,*N*′-bis-(Boc)-3-(2-pyrrolyl)alaninyl-serine methyl ester (*S*,*S*)-**15** had >96% diastereomeric purity. Hence, (2*S*)-3-(2-pyrrolyl)alanine **14** is considered to be of the same high enantiomeric purity.

(2*S*)-*tert*-Butyl (1-Hydroxy-4-oxo-oct-7-en-2-yl)carbamate (17) and *tert*-Butyl ((3*S*)-5-(But-3-en-1-yl)-5-hydroxytetrahydrofuran-3-yl)carbamate (18). In a flame-dried flask, under argon, CuCN (797 mg, 8.95 mmol) was treated with freshly prepared 1 M vinylmagnesium bromide in THF (96 mmol, 96 mL) at −45 °C, stirred for 30 min, treated via syringe over 10 min with a solution of methyl ester **16** (3.48 g, 14.92 mmol) in THF (30 mL), and stirred at −45 °C for 1 h. The cold bath was removed and replaced with an ice bath, and the mixture was stirred overnight during which time the bath had warmed to room temperature. The mixture was cooled to 0 °C, quenched with 1 M aqueous NaH_2PO_4 (250 mL), and shaken vigorously. The layers were separated. The aqueous phase was extracted with Et_2O (3 × 250 mL). The combined organic extracts were washed with a saturated $NaHCO_3$ solution (250 mL) and brine (250 mL), dried ($MgSO_4$), filtered, and evaporated to a residue, which was purified by silica gel column chromatography (30−50% ethyl acetate in hexane) to give acetal **18** as a yellow visqueous oil (695 mg, 18%) [TLC R_f 0.77 (50:50 EtOAc/hexane)] and homoallylic ketone **17** as a white solid (1.84 g, 48%) [TLC R_f 0.36 (50:50 EtOAc/hexane)]. Homoallylic ketones **17** and **18** were combined for the next reaction (2.53 g, 66%). In solution, **17** and **18** equilibrated and exhibited the following spectral data: ^{1}H NMR (300 MHz, CD_3Cl_3) δ 1.43 (1 s, 9H), 2.32 (q, *J* = 6.67, 14.12 Hz, 2H), 2.55 (t, *J* = 7.30 Hz,

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2H), 2.76 (d, J = 5.85 Hz, 2H), 3.67 (t, J = 5.10 Hz, 2H), 3.95 (m, 1H), 4.96−5.06 (m, 2H), 5.26 (br s, 1H), 5.79 (m, 1H).

(2*S*)-*N*-(Boc)-3-(2-Pyrrolyl)alaninol (20). 2,6-Lutidine (2.18 mL, 18.76 mmol), OsO_4 (2.5% in *tert*-butanol, 235.5 μL, 0.188 mmol), and $NaIO_4$ (8.02 g, 37.52 mmol) were added to a solution containing a mixture of homoallylic ketone **17** and acetal **18** (2.41 g, 9.38 mmol) in 3:1 dioxane/water (94 mL). The mixture was stirred at room temperature for 18 h and partitioned between H_2O (100 mL) and CH_2Cl_2 (200 mL). Then the aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), filtered, and evaporated to give aldehyde **19**: TLC R_f 0.57 (20:80 EtOAc/hexane). Without further purification, ketoaldehyde **19** (2.70 g crude) was treated with NH_4CO_2H (3.28 g, 52.05 mmol) and NaOAc/AcOH (2.70 g, 100 mol % w/w, prepared by mixing equimolar quantities of NaOAc and HOAc) in MeCN (260 mL) and heated at 65 °C until complete consumption of starting ketoaldehye **19** was observed by TLC [**19**: R_f 0.50 (5:95 EtOAc/chloroform)], and a new, less polar product was formed, **20** [R_f 0.57 (80:20 EtOAc/hexane)]. After 3 h, the mixture was cooled to room temperature and partitioned between pH 6.8 sodium phosphate buffer (200 mL) and a 2:1 Et_2O/CH_2Cl_2 solution (200 mL). The aqueous phase was extracted with 2:1 Et_2O/CH_2Cl_2 (2 × 200 mL). The combined organic layers were washed with brine (250 mL), dried ($MgSO_4$), filtered, and evaporated to a residue, which was purified by silica gel column chromatography (40−80% ethyl acetate in hexane) to give pyrrole **20** (813 mg, 36%) as a pale orange crystalline solid: mp 112−114 °C; TLC R_f 0.65 (80:20 EtOAc/hexane); $[\alpha]_D^{20}$ −14.46 (c 14.3 × 10^{-3}, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.88 (d, J = 6.4 Hz, 2H), 3.62 (d, J = 4.8 Hz, 2H), 3.81 (m, 1H), 4.93 (br d, J = 7.6 Hz, 1H), 5.98 (br s, 1H), 6.13 (dd, J = 2.8, 5.6 Hz, 1H), 6.70 (dd, J = 2.8, 4 Hz, 1H), 8.69 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 127.8, 117.5, 108.5, 107.2, 80.2, 64.4, 52.5, 29.9, 28.6; HRMS (ESI) calcd m/z for $C_{12}H_{20}N_2NaO_3$ [(M + Na)$^+$] 263.1366, found m/z 263.1367.

(2*S*)-*N*,*N*′,*O*-Tris-(Boc)-3-(2-pyrrolyl)alaninol (21). A solution of pyrrolylalaninol **20** (766.3 mg, 3.19 mmol) in MeCN (40 mL) at room temperature was treated with DMAP (47.8 mg, 0.306 mmol) and $(Boc)_2O$ (1.34 g, 6.12 mmol), stirred overnight, and evaporated to a residue, which was partitioned between H_2O (100 mL) and EtOAc (100 mL). The aqueous phase was extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with H_2O (100 mL) and brine (100 mL), dried (Na_2SO_4), filtered, and evaporated to give carbonate **21** (1.43 g, 80%) as a pale pink solid: mp 108−110 °C; TLC R_f 0.74 (40:60 EtOAc/hexane); $[\alpha]_D^{20}$ −22.14 (c 16.5 × 10^{-3}, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (s, 9H), 1.50 (s, 9H), 1.60 (s, 9H), 3.00−3.11 (m, 1H), 3.16 (dd, J = 4.6, 15 Hz, 1H), 4.08−4.21 (m, 3H), 4.86 (br d, J = 10.5 Hz, 1H), 6.04 (br s, 1H), 6.07 (t, J = 3.2 Hz, 1H), 7.19 (dd, J = 1.8, 3.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 153.7, 149.9, 131.5, 121.8, 113.7, 110.4, 83.9; HRMS (ESI) calcd m/z for $C_{22}H_{36}N_2NaO_7$ [(M + Na)$^+$] 463.2415, found m/z 463.2429.

(2*S*)-*N*,*N*′-Bis-(Boc)-3-(2-pyrrolyl)alaninol (13) from Carbonate (21). A solution of carbonate **21** (74 mg, 0.167 mmol) and K_2CO_3 (69 mg, 0.500 mmol) in MeOH (5 mL) was stirred at room temperature for 9 h. A solution of saturated $NaHCO_3$ (6 mL) was added to the mixture, which was extracted with EtOAc (3 × 12 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), filtered, and evaporated to give a residue, which was separated by column chromatography on silica gel (20−50% EtOAc in hexane). First to elute was carbonate **21** (9.8 mg) as a pale pink solid exhibiting identical characteristics as described above: TLC R_f 0.74 (40:60 EtOAc/hexane). Next to elute was (2*S*)-*N*,*O*-bis-(Boc)-3-(2-pyrrolyl)alaninol **22** (7.2 mg, 13%) as a pale yellow solid: mp 79−81 °C; TLC R_f 0.58 (40:60 EtOAc/hexane); $[\alpha]_D^{20}$ −9.13 (c 10.4 × 10^{-3}, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.52 (s, 9H), 2.86 (m, 2H), 3.95−4.09 (2 m partially overlapped, 2H), 4.09−4.17 (m, 1H), 4.81 (br s, 1H), 5.99 (br s, 1H), 6.13 (dd, J = 2.8, 6 Hz, 1H), 6.72 (dd, J = 2.6, 3.8 Hz, 1H), 8.67 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 154.0, 127.2, 117.7, 108.7, 107.8, 83.1, 80.1, 67.3, 49.9, 29.9, 28.6, 27.9; HRMS (ESI) calcd m/z for $C_{17}H_{29}N_2O_5$ [(M + H)$^+$] 341.2071, found m/z 341.2087; calcd m/z for $C_{17}H_{28}N_2NaO_5$ [(M + Na)$^+$] 363.1890, found m/z 363.1908. Next to elute was bis-(Boc)-(pyrrolyl)alaninol **13** (22.5 mg, 39%) as a pale yellow viscous oil [TLC R_f 0.26 (40:60 EtOAc/hexane)], followed by *N*-(Boc)-pyrrolylalaninol **20** (12 mg, 30%) as a pale orange crystalline solid [TLC R_f 0.13 (40:60 EtOAc/hexane)], both exhibiting identical characteristics as described above.

(*S*,*S*)-*N*,*N*′-Bis-(Boc)-3-(2-pyrrolyl)alaninyl-proline *N*″-Methylamide (23). A solution of amino acid **14** (50 mg, 0.141 mmol), HOBt (19.1 mg, 0.141 mg), DIEA (74 μL, 0.423 mmol), and proline *N*″-methylamide hydrochloride (46.4 mg, 0.282 mmol) in CH_2Cl_2 (7 mL) was stirred at 0 °C for 15 min and treated with TBTU (42.3 mg, 0.141 mmol). The ice bath was removed, and the mixture was allowed to warm to room temperature while being stirred. After 5 h, the volatiles were evaporated under vacuum. The residue was dissolved in EtOAc (20 mL), washed with saturated aqueous $NaHCO_3$ (2 × 7 mL), H_2O (5 mL), 1 N HCl (2 × 7 mL), H_2O (7 mL) and brine (7 mL), dried ($MgSO_4$), filtered, and evaporated to give dipeptide **23** as a white residue (59.3 mg, 91%): $[\alpha]_D^{20}$ −36.82 (c 16.3 × 10^{-3}, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) indicated a 30:70 ratio of amide *cis*- and *trans*-isomers N-terminal to proline, δ 1.37 (s, 6H) [1.39 (s, 2H)] [1.59 (s, 3.6H)] 1.60 (s, 6.4H), 1.78−1.87 (m, 1.3H), 1.92−1.98 (m, 1.4H), 2.35−240 (m, 0.7H) [2.45−2.48 (m, 0.3H)], 2.72−2.77 (2 d partially overlapped, 3H), 3.10−3.22 (m, 1.2H), 3.26 (dd, J = 6.7, 14.5 Hz, 0.8H), 3.40−3.45 (m, 0.6H), 3.50−3.54 (m, 0.6H), 3.72 (q, J = 8.8, 17.7 Hz, 0.7H), [4.25 (d, J = 7 Hz, 0.3H)], 4.6 (d, J = 7.8 Hz, 0.7H), [4.53 (q, J = 6.3, 14 Hz, 0.3H)], 4.80 (q, J = 7.3, 15.5 Hz, 0.6H), [5.12 (d, J = 5.7 Hz, 0.3H)], 5.20 (d, J = 8.9 Hz, 0.6H), 6.02 (s, 0.7H), 6.04−6.09 (m, 1.3H), 6.67 (br s, 0.6H) [7.64 (br s, 0.2H)], 7.18−7.22 (2 m partially overlapped, 1H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 172.8, 171.5, (156.0) 155.3, 149.6, 130.0 (129.2), (122.3) 122.2, (115.0) 114.3, (110.4) 110.2, (84.7) 84.4, (80.2) 79.8, (60.7) 60.1, (52.5) 51.7, 47.3 (46.9), 32.3, (31.1) 31.0, 28.5 (28.4), 28.1, (27.3) 26.3, 25.1 (22.2); HRMS (ESI) calcd m/z for $C_{23}H_{37}N_4O_6$ [(M + H)$^+$] 465.2708, found m/z 465.2723; calcd m/z for $C_{23}H_{36}N_4NaO_6$ [(M + Na)$^+$] 487.2527, found m/z 487.2537.

(*S*,*S*)-*N*-Acetyl-*N*′-(Boc)-3-(2-pyrrolyl)alaninyl-proline *N*″-Methylamide (24). A solution of *N*,*N*′-bis-(Boc)-dipeptide *N*″-methyl amide **23** (24.1 mg, 0.052 mmol) in 1:3 TFA/CH_2Cl_2 (3 mL) was stirred at room temperature for 30 min and evaporated on a rotary evaporator. The resulting dipeptide *N*″-methyl amide trifluoroacetate was dissolved in CH_2Cl_2 (3 mL), treated with K_2CO_3 (71.9 mg, 0.520 mmol) and Ac_2O (49 μL, 0.520 mmol), stirred for 18 h, filtered, washed with CH_2Cl_2 (2 × 10 mL), and evaporated to give *N*-acetyl-*N*′-Boc-dipeptide *N*″-methyl amide **24** (19.3 mg, 92%) as a colorless residue: TLC R_f 0.13 (5:95 MeOH/dichloromethane); $[\alpha]_D^{20}$ −22.33 (c 15.5 × 10^{-3}, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) indicated a 35:65 ratio of amide *cis*- and *trans*-isomers N-terminal to proline δ [1.58 (s, 3H)], 1.60 (s, 6H), 1.81−1.88 (m, 1.5H), 1.91 (s, 2H) [1.94 (s, 1H)], 1.95−2.02 (m, 1.5H), 2.34−2.38 (m, 0.7H), 2.47−2.52 (m, 0.3H), 2.75 (d, J = 4.9 Hz, 2H) [2.77 (d, J = 4.2 Hz, 1H)], 3.11−3.17 (m, 1H), [3.25 (dd, J = 5.6, 14 Hz, 0.3H)] 3.33 (dd, J = 5.9, 14.3 Hz, 0.7H), 3.48−3.57 (m, 1.3H), 3.80 (m, 0.7H), [4.34 (d, J = 7.7 Hz, 0.3H] 4.56 (dd, J = 2.1, 8.4 Hz, 0.7H), [4.52 (m, 0.3H)] 0.508 (m, 0.7H), 6.0 (m, 0.6H), 6.05−6.09 (m, 1.4H), 6.26−6.31 (2 br d partially overlapped, 1H), 6.58 (br d, J = 4.2 Hz, 0.7H) [7.73 (br d, J = 4.9 Hz, 0.3H), 7.18−7.20 (2 m partially overlapped, 1H); ^{13}C NMR (175 MHz, $CDCl_3$) δ (172.4) 171.4, 171.0 (170.9), (170.8) 169.8, (149.7) 149.6, 129.9 (129.2), (122.4) 122.3, (115.0) 114.4, (110.5) 110.1, (84.8) 84.4, (60.7) 60.2, (52.2) 50.5, 47.4 (46.9), 31.9 (31.1), (30.5) 29.8, (28.1) 28.0, (27.4) 26.3, (26.6) 25.1, 23.1 (22.9); HRMS (ESI) calcd m/z for $C_{20}H_{31}N_4O_5$ [(M + H)$^+$] 407.2289, found m/z 407.2308; calcd m/z for $C_{20}H_{30}N_4NaO_5$ [(M + Na)$^+$] 429.2108, found m/z 429.2124.

***N*-Acetyl-3-(2-pyrrolyl)alaninyl-proline *N*″-Methylamide (25).** *N*-Acetyl-*N*′-Boc-dipeptide *N*″-methyl amide **24** (3.8 mg, 0.009 mmol) in 1,2-dichlorobenzene was heated at 180 °C for 30 min. The mixture was cooled to room temperature and purified by silica gel column chromatography (3−5% MeOH in dichloromethane) to give *N*-acetyl-dipeptide *N*″-methyl amide **25** as a yellow residue (2 mg,

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70%): TLC R_f 0.16 (5:95 MeOH/dichloromethane); $[\alpha]_D^{20}$ −32.22 (*c* 5.33 × 10^{-3}, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) indicated only the *trans*-isomer δ 1.81−2.17 (m, 7 H), 2.86 (d, *J* = 4.8 Hz, 3 H), 2.96−3.07 (m, 2 H), 3.14 (dd, *J* = 3.4, 14.6 Hz, 1 H), 3.54−3.62 (m, 1 H), 4.31 (dd, *J* = 5.4, 8.2 Hz, 1 H), 4.86 (m, 1 H), 6.00 (m, 1 H), 6.06−6.16 (2 m overlapped, 2 H), 3.78 (br d, *J* = 8 Hz, 1 H), 6.72 (m, 1 H), 9.91 (br s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 171.0, 169.7, 125.6, 118.4, 108.3, 108.0, 61.0, 51.7, 47.5, 31.5, 29.7, 26.7, 25.5, 23.5; HRMS (ESI) calcd *m*/*z* for for $C_{15}H_{22}N_4NaO_3$ $[(M + Na)^+]$ 329.1584, found *m*/*z* 329.1591.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR, NOESY, and TOCSY spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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