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Synthesis and conformational properties of model dipeptides containing novel axially chiral α,β-didehydroamino acids at the (*i*+1) position of a β-turn conformation

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Abstract—A series of model dipeptides containing some novel axially chiral α,β-didehydroamino acids at the (*i*+1) position has been synthesised by reaction of the corresponding 4-(4-alkylcyclohexylidene)-2-phenyl-1,3-oxazol-5(4*H*)-one with (*S*)-phenylalanine cyclohexylamide. The conformations of two dipeptides in the crystal state have been studied by X-ray diffraction crystallographic analysis. The backbone torsion angles suggest that both peptides adopt similar type-II′ β-turn conformations. NMR spectroscopy has revealed that relatively rigid β-turn structures also persist in solution and that the absolute configurations of the axially chiral α,β-didehydroamino acids do not significantly influence the conformation of the peptide chain. Both heterochiral and homochiral dipeptides are found to accommodate the same βII′-turn conformation. Axially chiral α,β-didehydroamino acids (R_a)- and (S_a)-4-methyl-, 4-phenyl- and (4-*tert*-butylcyclohexylidene)glycine can be considered as elongated structural analogues of alanine, phenylglycine and *tert*-leucine of *R* and *S* configuration since, in these chiral α,β-didehydroamino acids, the methyl, phenyl and *tert*-butyl groups are located about 4.3 Å away from the peptide backbone in which they are incorporated.

1. Introduction

Non-coded α-amino acids with unusual side chains can exhibit peculiar properties once introduced into peptides. Such systems now represent some of the most important areas of research in the fields of organic chemistry, medicinal chemistry, and protein engineering.[1] Among non-proteinogenic α-amino acids, the α,β-didehydroamino acids have recently received a great deal of attention.[2] These compounds are present in many naturally occurring peptides that show important biological activities.[3] For example, didehydroalanine, the most widely observed natural α,β-unsaturated amino acid, has been identified as a constituent of a substantial number of cyclic peptides produced by microorganisms, including the antibiotics nisin, mersacidin, subtilin[4] and phytotoxic AM-toxins.[5] This compound has also been found in some enzymes, for example, phenylalanine ammonia lyase from plants,[6] which are formed by post-translational dehydration of serine residues.

One of the major goals in modern amino acid chemistry is to develop conformationally restricted amino acids that, after incorporation into peptides, may give rise to enhanced biological activity by decreasing the degree of freedom of the peptide. Whereas extensive efforts have been devoted to the design of peptides with well defined backbone conformations,[7] the geometry of the side chain in amino acid moieties has received considerable less attention despite side chains are directly involved in the molecular recognition processes which depend on their adequate spatial disposition.[8]

Structurally, α,β-didehydroamino acid residues are distinguished by forming, upon incorporation into a peptide chain, a system involving three rigid groups located on the C^{α} atom: the α,β-double bond flanked by two adjacent amide bonds. Consequently, these amino acids have a powerful rigidifying effect on the peptide backbone and effectively restrict the orientation of the side chain. The orientation of the β-substituents is fixed by the stereochemistry of the double bond which offers the possibility to

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evaluate accurately the influence of the side chain three-dimensional arrangement. For this reason these amino acids have been used for the modification of bioactive peptide sequences in order to obtain highly active agonist or antagonist analogues and indeed this modification has become one of the most promising ways to establish structure–bioactivity relationships.[9] Another feature of didehydropeptides is the increased stability to degradative enzymes, which has led to synthetic enzyme inhibitors that act as non-hydrolyzable substrate mimics.[10] These features have been the driving force for the increased interest in the design of didehydropeptide-based therapeutic agents.

As pointed out above, the presence of the sp^2 hybridised α-carbon atom, the extended conjugation on account of the π system, the restriction of the peptide backbone and the specific orientation of the side chain due to the double bond are some of the special features that make didehydropeptides attractive targets for conformational studies.[11] Most such studies have been limited to model synthetic peptides containing α,β-didehydroamino acids derived from standard (proteinogenic) saturated residues, mainly didehydrophenylalanine (ΔPhe). X-ray diffraction analyses, conformational energy calculations, NMR, and CD have largely been used to confirm the presence of ordered structures in such peptides both in the crystal state and in solution.[12] The available data, most concerning systems with the α,β-didehydroamino acid located at the $(i+2)$ position, indicate that β-turns[13] are often stabilised in short sequences, whereas helix formation may be promoted in longer peptides. For example, it has been clearly established that a didehydrophenylalanine residue at this position gives rise to a type-II β-turn conformation.

On the other hand, the number of available structures with a α,β-didehydroamino acid at the $(i+1)$ position remains very small. Thus, a clear picture has not yet emerged in terms of preferred conformations and more sequences with the α,β-didehydroamino acid at the $(i+1)$ position need to be analysed.

2. Results and discussion

A preliminary account of the results described here has been published[14] and dealt with the synthesis, isolation and characterisation of dipeptides PhCO-(R_a)-(CH_3Cy)Gly-(*S*)-Phe-NHCy and PhCO-(S_a)-(CH_3Cy)Gly-(*S*)-Phe-NHCy [(R_a,*S*)- and (S_a,*S*)-**4a**], where (CH_3Cy)Gly stands for (4-methylcyclohexylidene)glycine. Both of these systems contain an axially chiral dehydroamino acid at the $(i+1)$ position. To the best of our knowledge, that preliminary communication described the first examples of chiral α,β-didehydroamino acids as structural features of α,β-didehydroamino acids preclude the existence of a stereogenic centre. In the present paper, we report in detail the preparation of the two aforementioned model dipeptides and four structural analogues: PhCO-(R_a)-(PhCy)Gly-(*S*)-Phe-NHCy [(R_a,*S*)-**4b**], PhCO-(S_a)-(PhCy)Gly-(*S*)-Phe-NHCy [(S_a,*S*)-**4b**], PhCO-(R_a)-(^{t}BuCy)Gly-(*S*)-Phe-NHCy [(R_a,*S*)-**4c**], and PhCO-(S_a)-(^{t}BuCy)Gly-(*S*)-Phe-NHCy [(S_a,*S*)-**4c**], where (PhCy)Gly and (^{t}BuCy)Gly represent α,β-didehydroamino acids (4-phenylcyclohexylidene)glycine and (4-*tert*-butylcyclohexylidene)glycine, respectively.

Bearing in mind that model dipeptides RCO-X_{aa}-Y_{aa}-NHR′ are the most simple molecules that are compatible with β-turn folding, compounds (R_a,*S*)-**4a–c** and (S_a,*S*)-**4a–c** would be expected to allow the study of the conformational preferences induced by this kind of α,β-didehydroamino acid when incorporated at the $(i+1)$ position of a model dipeptide and enable analysis of how the absolute configuration of axially chiral residues affects the folding mode of the compound.

Conformational analyses of the six model dipeptides in solution were performed by ^{1}H NMR spectroscopy. The isolation of crystalline samples of compounds PhCO-(R_a)-(CH_3Cy)Gly-(*S*)-Phe-NHCy [(R_a,*S*)-**4a**] and PhCO-(R_a)-(^{t}BuCy)Gly-(*S*)-Phe-NHCy [(R_a,*S*)-**4c**] allowed their analysis in the crystal state by X-ray diffraction.

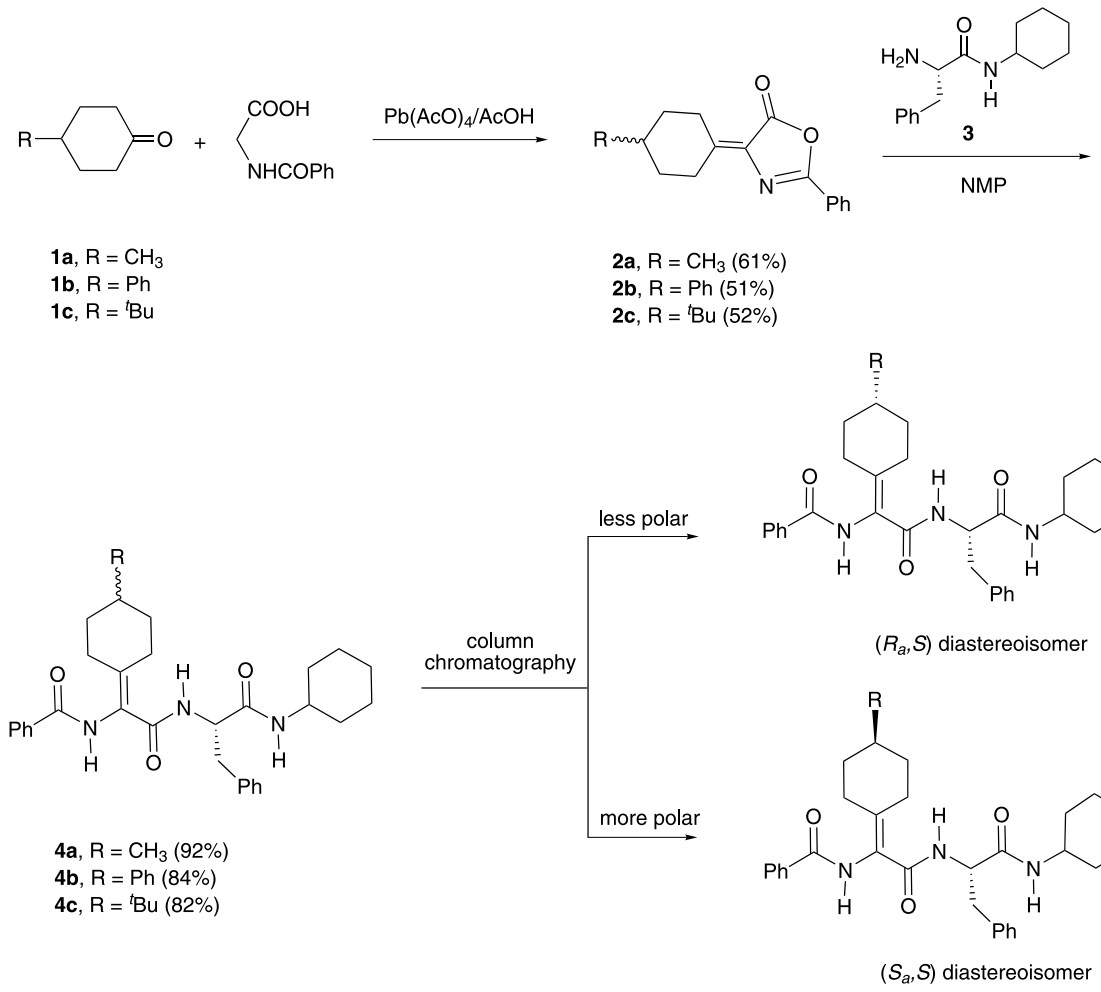
2.1. Synthesis of compounds (R_a,*S*)-4a–c and (S_a,*S*)-4a–c

Compounds (R_a,*S*)-**4a–c** and (S_a,*S*)-**4a–c** were synthesised by the 'oxazolone method' outlined in Scheme 1. This strategy was developed by Obrecht et al.[15] to prepare enantiomerically pure C^{α}-tetrasubstituted amino acids using (*S*)-phenylalanine cyclohexylamide as a chiral resolving agent. In our case, the synthesis started with the condensation of three different 4-alkylcyclohexanones (**1a–c**) with hippuric acid under typical Erlenmeyer conditions to give the required 4-(4-alkylcyclohexylidene)-2-phenyl-1,3-oxazol-5(4*H*)-ones (**2a–c**) as racemic mixtures in moderate yields (50–60%). Coupling of these compounds with (*S*)-phenylalanine cyclohexylamide (**3**) at 90 °C using *N*-methylpyrrolidin-2-one (NMP) as solvent cleanly afforded the corresponding peptides **4a–c** as equimolecular mixtures of diastereoisomers in high yields. Careful column chromatography on silica gel, using methylene chloride/ethyl acetate (3/1) as the eluent, gave analytically pure samples of both (R_a,*S*) and (S_a,*S*) diastereoisomers from each mixture of compounds.

The configuration of the less polar diastereoisomer of compounds **4a** and **4c** was unambiguously assigned by single crystal X-ray diffraction analysis based on the known (*S*)-configuration of phenylalanine. This analysis established the absolute configuration of the corresponding chiral α,β-didehydroamino acid residues as R_a in both compounds. The assignment of the S_a configuration for the more strongly retained diastereoisomers of compounds **4a** and **4c** was deduced by exclusion. Unfortunately, all efforts to crystallise the two diastereoisomers of dipeptide **4b** were unsuccessful. Thus, the absolute configurations (R_a,*S*) and (S_a,*S*) were assumed for the less polar diastereoisomer and the more polar one, respectively. This assumption is supported by the similarity in the spectroscopic behaviour of these compounds and those of peptides (R_a,*S*)-**4a** and **4c** and (S_a,*S*)-**4a** and **4c**, whose absolute configurations were known.

2.2. Crystal structures

Dipeptides (R_a,*S*)-**4a** and (R_a,*S*)-**4c** gave suitable crystals for



Scheme 1. Synthesis of compounds (R_a,*S*)-**4a**–**c** and (S_a,*S*)-**4a**–**c** according to the 'oxazolone method'.

analysis by X-ray diffraction. The peptide (R_a,*S*)-**4a** crystallised with two independent molecules, A and B, in the asymmetric unit. These two independent molecules differ slightly in their conformation. The molecular structures of the two compounds, along with the atomic numbering schemes, are illustrated in Figures 1 and 2, respectively. Relevant backbone and side-chain torsion angles[16] are given in Table 1. Table 2 contains the intra- and intermolecular H-bond parameters.

With the exception of the α,β-didehydroamino acid residues, bond lengths and angles in (R_a,*S*)-**4a** and

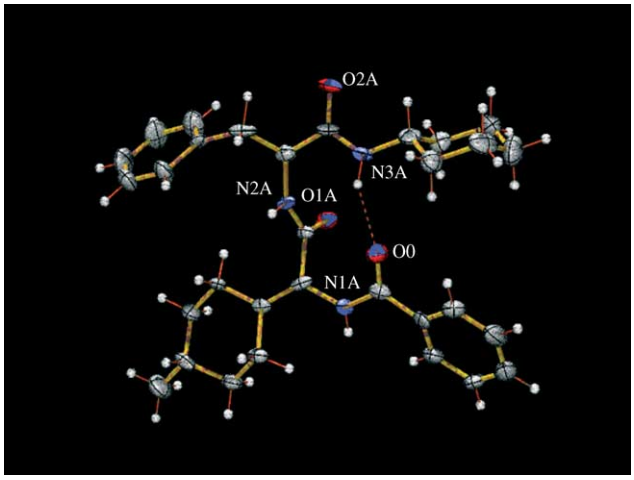


Figure 1. X-ray diffraction structure of compound (R_a,*S*)-**4a** (independent molecule A). Ellipsoids are shown at the 50% probability level. The intramolecular H-bond is represented by dashed lines.

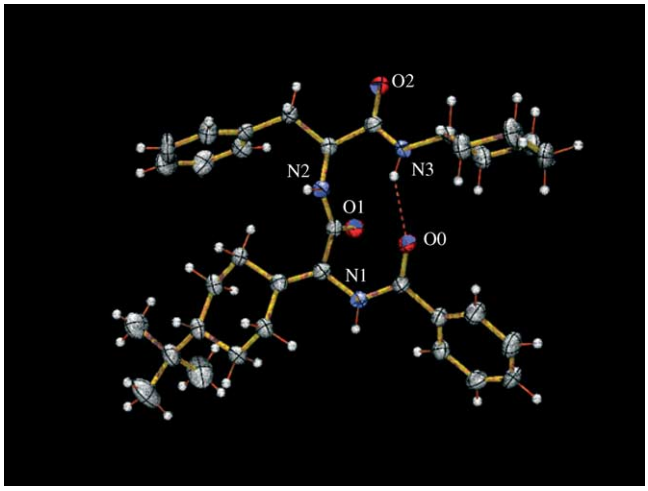


Figure 2. X-ray diffraction structure of compound (R_a,*S*)-**4c**. Ellipsoids are shown at the 50% probability level. The intramolecular H-bond is represented by a dashed line.

Table 1. Selected torsion angles (deg) for the dipeptides (R_a,*S*)-**4a** and (R_a,*S*)-**4c**

Torsion angle	(R_a,*S*)-**4a** (mol A/mol B)	(R_a,*S*)-**4c**
ω_0	171.5(9)/174.1(9)	−171.3(4)
ϕ_1	51.2(13)/48.0(13)	38.7(6)
ψ_1	−122.5(9)/−124.7(9)	−126.1(4)
ω_1	−179.1(9)/−178.3(9)	−176.7(4)
ϕ_2	−87.5(12)/−83.8(12)	−89.9(5)
ψ_2	6.5(15)/4.7(14)	9.4(6)
ω_2	170.1(10)/171.6(9)	175.0(4)
$\chi_1^{1,1}$	−170.6(9)/−171.1(10)	−166.5(4)
$\chi_1^{1,2}$	8.9(15)/7.0(17)	5.0(7)
χ_2^1	−73.1(12)/−76.2(12)	−59.0(6)

(R_a,*S*)-**4c** are in good agreement with those found in usual peptides.[17] The average C^{α}=C^{β} bond length in (CH_3-Cy)Gly and (^{t}BuCy)Gly residues from crystal structure data is 1.35 Å, which is slightly larger than the value found in other unsaturated residues.[11b] The C^{α}–C bond length (1.50 Å) is somewhat shorter (by 0.03 Å) than that in saturated amino acid residues (1.53 Å).[17] The N–C^{α} bond distance is 1.43 Å, again also slightly less than the corresponding distance of 1.45 Å in saturated amino acid residues. The values of the N–C^{α}–C and $C^{\gamma 1}$–C^{β}–$C^{\gamma 2}$ bond angles of 114.1 and 113.2°, respectively, are less than the standard value, 120°, from an sp^2 hybridised carbon atom, whereas the values of N–C^{α}=C^{β}, C–C^{α}=C^{β}, $C^{\gamma 1}$–C^{β}=C^{α} and $C^{\gamma 2}$–C^{β}=C^{α} are larger (between 123.8 and 122.4°).

Both model dipeptides exhibit interesting aspects of peptide folding. The similarity of the conformations is clearly shown by the torsion angles of the dipeptide backbone (Table 1). All amide bonds have the *trans*-conformation ($\omega = \pm 170$–179) and the ϕ/ψ-combinations for amino acids $i+1$ and $i+2$ are close to the ideal values for type II′ β-turn conformations (60/−120, −80/0). In (R_a,*S*)-**4a** the mean deviation from these values is ±6.3°, with the highest deviation being −12° for the torsion angle ϕ_1 in molecule B. In the case of (R_a,*S*)-**4c**, which contains the bulkier *tert*-butyl substituent in the cyclohexane ring, all backbone torsion angles show larger deviations from ideal values, with the mean deviation being ±11.7°. In each compound, the molecular conformation is stabilised by an intramolecular C=O···H–N hydrogen bond that links two termini of the backbone chain to form a 10-membered ring (Table 2), which is typical for a β-turn conformation.

The side-chain torsion angles indicate that both structures have the benzyl side chain of the (*S*)-phenylalanine residue in a (−)-*syn*-clinal disposition that allows the central amide group to be engaged in intermolecular hydrogen bonds. The torsion angles $\chi_1^{1,1}$ and $\chi_1^{1,2}$ are centered around 0° but deviations from this value are considerable, even rising to 13.5°. This information indicates that three side-chain atoms (C^{β}, $C^{\gamma 1}$ and $C^{\gamma 2}$) and three backbone atoms (N, C^{α}, C′) of the α,β-didehydroamino acid residues are not coplanar. Methyl and *tert*-butyl substituents at the 4-position of the cyclohexane ring are found in the equatorial position. Cyclohexylidene moieties adopt a distorted chair conformation.

Considering the relative spatial dispositions of the side chains in both crystalline compounds, it is found that 4-alkylcyclohexylidene and benzyl side chains are situated in nearly orthogonally planes. The N-terminal benzoyl and the C-terminal cyclohexylamino moieties extend in the opposite direction and are also situated in nearly orthogonally planes. With this arrangement of the hydrophobic groups, the molecules can pack in two dimensional layers parallel to the *z*-axis and expose tightly packed arrays of phenyl, benzyl, 4-alkylcyclohexylidene and cyclohexyl groups on either side, thus forming relatively smooth hydrophobic contact surfaces between adjacent layers. The hydrophobic surfaces of the layers are devoid of marked cavities that could provide possibilities for tight side-chain interlocking. Within each layer, there are two orthogonal H-bond networks with all amide units H-bonded (Table 2). The first network is established by intermolecular H-bonds between the central and C-terminal peptide units. The second one is formed by above-mentioned intramolecular H-bonds and intermolecular H-bonds between the N-terminal and central peptides units, in crystal packing of dipeptide (R_a,*S*)-**4a**, or between the N-terminal peptide unit, a molecule of solvent (methanol) and the central peptide unit, in crystal packing of dipeptide (R_a,*S*)-**4c**. In the second case, the presence of a solvent molecule in the asymmetric unit provides the opportunity for the formation of additional intermolecular H-bonds. N1–H1 donates an H-bond to the CH_3OH molecule, which in turn acts as a donor for another H-bond to O1. Thus, the CH_3OH molecule is H-bonded to two dipeptide molecules.

2.3. Structures in solution

Information about the three dimensional structures of compounds (R_a,*S*)-**4a–c** and (S_a,*S*)-**4a–c** can be obtained from NMR measurements. In these structures, intramolecular hydrogen bonding may provide the principal

Table 2. Intra- and intermolecular H-bond parameters for the dipeptides (R_a,*S*)-**4a** and (R_a,*S*)-**4c**

Dipeptide	Donor (D–H)	Acceptor (A)	Symmetry operation	Distance D…A (Å)	Distance H…A (Å)	Angle D–H…A (deg)
(R_a,*S*)-**4a**	N1A–H1A	O1A	$1-x, 1/2+y, -1-z$	3.166(11)	2.30	168.3
	N2A–H2A	O2A	$2-x, 1/2+y, -1-z$	3.068(11)	2.20	169.4
	N3A–H3A	O0A	x, y, z	2.866(12)	2.05	153.9
	N1B–H1B	O1B	$2-x, -1/2+y, -2-z$	3.187(11)	2.31	170.9
	N2B–H2B	O2B	$1-x, -1/2+y, -2-z$	3.008(12)	2.14	167.6
	N3B–H3B	O0B	x, y, z	2.851(11)	2.04	152.8
(R_a,*S*)-**4c**	N1–H1	OM	$-x, -1/2+y, 1-z$	2.965(5)	2.10	166.2
	N2–H2	O2	$1-x, -1/2+y, 1-z$	2.929(5)	2.07	166.8
	N3–H3	O0	x, y, z	2.865(5)	2.06	151.4
	OM–HOM	O1	x, y, z	2.766(5)	1.94	169.3

Table 3. Most significant NMR parameters for compounds (R_a,*S*) and (S_a,*S*)-**4a–c** in $CDCl_3$ solution

NMR parameter	(R_a,*S*)-**4a**	(S_a,*S*)-**4a**	(R_a,*S*)-**4b**	(S_a,*S*)-**4b**	(R_a,*S*)-**4c**	(S_a,*S*)-**4c**
δ-NHCy[a]	7.27	7.58	7.23	7.65	7.25	7.60
δ-NHPhe[a]	6.05	6.04	6.13	6.11	6.03	6.02
δ-NHBz[a]	8.26	8.91	7.91	9.20	8.00	8.89
$^3J^{\alpha}_{NH-CH}$(Phe)[b]	8.50	8.90	8.70	8.90	8.60	8.70

[a] ppm.
[b] Hz.

driving force for turn conformation. For this reason, experimental investigations were carried out in a relatively non-polar solvent ($CDCl_3$), which does not offer strong hydrogen-bonding competition in 10 mM solutions.

Well-resolved 300 MHz ^{1}H NMR spectra were obtained for all compounds examined in this work. These spectra allowed completely unambiguous assignment of most CH proton resonances and all NH proton resonances. In $CDCl_3$ solution the benzoylamino group NH proton resonances appeared as downfield singlets (8–9 ppm), a situation that allowed their straightforward assignment because amide proton resonances in conjugation with the double bond system in α,β-didehydroamino acid derivatives have often been observed downfield with respect to other NH resonances. The cyclohexylamide NH and phenylalanine NH protons appeared as doublets and were unambiguously assigned by two-dimensional correlated spectroscopy (COSY), which showed that the phenylalanine NH proton resonance always appears at a higher field than the cyclohexylamide NH proton resonance. Table 3 contains the chemical shifts of the amido NH protons in 10 mM solutions in $CDCl_3$ for all compounds at 293 K.

Insights into the nature of hydrogen bonding can be gained from NMR data in relation to different criteria: chemical shifts of the amide NH protons, concentration dependence of the ^{1}H NMR chemical shifts of amide protons, amide proton–deuterium exchange rate, solvent perturbation of the amide proton signal upon addition of an H-bonding acceptor solvent (DMSO-d_6), paramagnetic radical induced line broadening of NH proton resonances caused by the addition of the free radical TEMPO, and temperature dependence of the ^{1}H NMR chemical shifts of amide protons.[18] In the following discussion we considered the chemical shifts of the amide NH protons, and the effect of the addition of increasing amounts of the H-bonding acceptor solvent (DMSO-d_6) to a 10 mM solution of the corresponding dipeptide in $CDCl_3$ over the range from 0 to 10%. Figure 3 shows the effects of the added perturbing agent on the NH proton resonances in compounds (R_a,*S*)- and (S_a,*S*)-**4a–c** in $CDCl_3$ solution.

In 10 mM $CDCl_3$ solution, the benzoylamino NH proton resonances of compounds (R_a,*S*)- and (S_a,*S*)-**4a–c** appeared downfield ($\delta > 8.5$ ppm) and an appreciable downfield shift is observed upon the addition of increasing amounts of the H-bonding acceptor solvent (DMSO-d_6) to a 10 mM of the corresponding dipeptide in $CDCl_3$. These data suggest that benzamido protons are not involved in intramolecularly hydrogen-bonded states.

In all model dipeptides the following ^{1}H NMR parameters were observed for the NH proton of the cyclohexylamide group. A chemical shift of about 7.5 ppm, which is downfield by about 0.5 ppm relative to the corresponding amide proton in the cyclohexylamide unit of phenylalanine, and a reduced solvent perturbation upon addition of increasing amounts of DMSO-d_6 [δ(NH) 0.1–0.25 ppm] were observed. This behaviour is consistent with the presence of a significant population of molecules in which these protons are involved in the formation of a stable intramolecular hydrogen bond.

The resonance of the NH proton signal of phenylalanine appears at about 6 ppm, a chemical shift characteristic of a peptide backbone proton that is not involved in hydrogen bonding. In all model dipeptides the addition of increasing

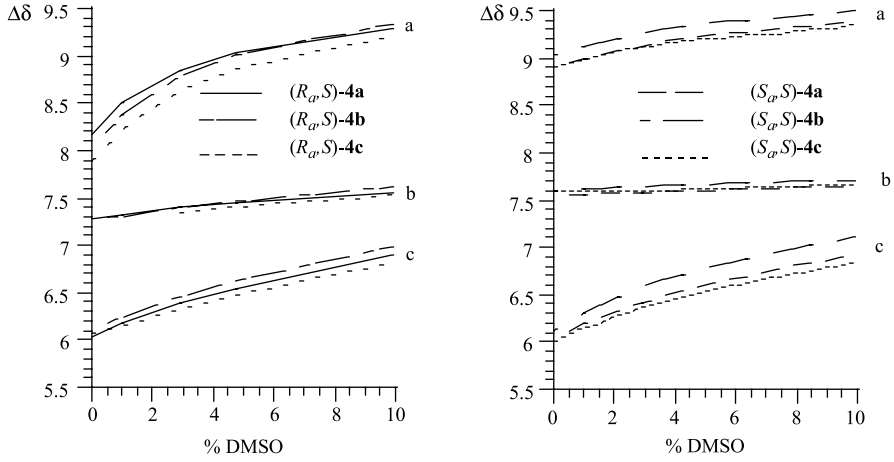


Figure 3. Plot of NH proton chemical shifts in the ^{1}H NMR spectra as a function of increasing % of DMSO added to the $CDCl_3$ solution for (a) NHCOPh, (b) NHCOCy and (c) NHPhe protons.

Table 4. Most significant results observed for compounds (R_a,*S*)- and (S_a,*S*)-**4a–c** in conventional 1D-difference NOE experiments carried out in 10 mM $CDCl_3$ solutions at 293 K

Spin *i* identity	Spin *j* identity	$\eta i(j)$ [$\eta j(i)$]×100					
		(R_a,*S*)-**4a**	(S_a,*S*)-**4a**	(R_a,*S*)-**4b**	(S_a,*S*)-**4b**	(R_a,*S*)-**4c**	(S_a,*S*)-**4c**
NHCOPh	$H^{\gamma'}$[(RCy)Gly]	7.5(10.9)	11.0(12.1)	6.2(n.d.)	3.2(2.2)	8.9(14.6)	9.9(14.2)
NHPhe	H^{γ}[(RCy)Gly]	3.4(6.5)	4.8(7.1)	2.3(4.2)	1.4(4.5)	3.9(6.9)	3.2(8.0)
NHPhe	H^{α}(Phe)	6.4(2.5)	6.1(1.9)	3.5(2.4)	3.1(1.8)	6.1(2.4)	6.7(2.0)
NHPhe	$H^{\beta h}$(Phe)	3.5(4.7)	3.0(3.7)	−(2.1)	1.3(2.9)	n.r.[a]	3.0(6.1)
H^{α}(Phe)	NHCy	7.3	7.2	n.r.[a]	2.5	n.r.[a]	6.8
H^{α}(Phe)	$H^{\beta l}$(Phe)	5.4(16.5)	4.3(17.5)	1.8(7.7)	2.8(8.1)	n.r.[a]	4.0(25.9)

h and l superscripts indicate β proton to higher or lower field, respectively.
[a] Signals due to this proton were not resolved to allow presaturation.

amounts of the H-bonding acceptor solvent (DMSO-d_6) to a 10 mM solution of the corresponding dipeptide in $CDCl_3$ resulted in appreciable downfield shifts of the phenylalanine NH ($\Delta\delta$ 0.8–1 ppm) resonances. These observations are characteristic of a peptide backbone proton that is not involved in hydrogen bonding.

It is worth mentioning that in homochiral compounds (S_a,*S*)-**4a–c** the benzoylamino NH proton signal is shifted to a lesser extent upon addition of DMSO-d_6 than the same signal in heterochiral compounds (R_a,*S*)-**4a–c**. This situation could be indicative of a relative shielding effect from the solvent in homochiral compounds due to the position of this NH within the β-turn. On the other hand in $CDCl_3$ solution the benzoylamino NH proton signal in heterochiral compounds (R_a,*S*)-**4a–c** is more shielded than the same signal in homochiral compounds (S_a,*S*)-**4a–c**.

All the above data are consistent with the occurrence of a significant population of β-turn conformers stabilised by an intramolecular hydrogen bond of the $i \leftarrow i+3$ type residues for compounds (R_a,*S*)- and (S_a,*S*)-**4a–c** in solution.

Of all the NMR parameters, coupling constants can be fruitfully used in determining spatial orientations of the interacting nuclei. For the phenylalanine residue, the vicinal ^1H–^1H coupling constant for groupings H–N–C–H^{α} (J^{α}_{NH-CH}) and H^{α}–C–C–H^{β} ($J_{\alpha\beta}$) could be elucidated by analysing the NH, H^{α} and H^{β} signals simultaneously.

The vicinal coupling constants J^{α}_{NH-CH} can be related to the torsion angle around the NH–CH^{α} Φ by a Karplus-type equation and, among the different sets of coefficients described in the literature for this type of equation, we chose that proposed by Cung et al.[19]

$$J^{\alpha}_{NH-CH} = 8.6\cos^2(\Phi \pm 60) - 2.9\cos(\Phi \pm 60)$$

[(+) for D and *R* configurations and (−) for L and *S* configurations, respectively]

According to this equation a J^{α}_{NH-CH} value of 8.50–8.90 H is indicative of a dihedral angle Φ, defined as recommended by the IUPAC-IUB commission,[16] of about −90°, as one would expect for the residue $i+2$ of a type II′ or a type I β-turn.

The best means of obtaining direct evidence for the predominance of a particular β-turn conformation in solution is from NOE experiments. This is because the observation of a direct $^1H\{^1H\}$NOE between a pair of protons is evidence for a significant population of conformers in which the protons are close, typically within 3–3.5 Å for flexible small peptides in solution.[20] Difference NOE experiments were carried out by irradiation of various X–H protons in compounds (R_a,*S*)-**4a–c** and (S_a,*S*)-**4a–c** in 10 mM $CDCl_3$ solutions at 293 K. A summary of the results obtained upon irradiation of these signals is shown in Table 4

A small but reproducible NOE was observed between the $H^{\gamma}_{(i+1)}$ [(RCy)Gly] and the phenylalanine NH protons for compounds (R_a,*S*)-**4a–c** and (S_a,*S*)-**4a–c** (see Table 4), which suggests short distances (<3.5 Å) between these nuclei. The interatomic distances between H^{γ} on the residue $i+1$ and the phenylalanine NH protons mainly depend on the absolute configuration of the chiral axis (R_a or S_a) and the ϕ and ψ torsion angles of the central residues, that is, on the type of turn adopted by the main chain. Molecular mechanics calculations were performed to ascertain the interatomic distances[21] between both *axial* and *equatorial* protons occupying this position and the phenylalanine NH proton in energy-minimised structures. Ideal β-turn conformations of types I and II′ were adopted and these show that this condition is only fulfilled if the studied compounds adopt a type II′ β-turn. In this case the existence of NOE between the $H^{\gamma}_{(i+1)}$ [(RCy)Gly] and the phenylalanine NH protons for compounds (R_a,*S*)-**4a–c** and (S_a,*S*)-**4a–c** proves that these compounds adopt a type II′ β-turn conformation in $CDCl_3$ solution. The calculated values for ideal β-turn conformations for compounds (R_a,*S*)-**4c** and (S_a,*S*)-**4c** are given in Table 5.

Table 5. Interatomic distances[a] (MM) between the $H^{\gamma}_{(i+1)}$ [(RCy)Gly] and the Phe($i+1$) NH proton calculated for ideal β-turn conformations of compounds (R_a,*S*)-**4c** and (S_a,*S*)-**4c**

	βI	βII′
H^{γ}_{eq}···NHPhe [(R_a,*S*)-**4c**]	4.39	2.63
H^{γ}_{ax}···NHPhe [(R_a,*S*)-**4c**]	5.21	2.68
NHBz···NHPhe [(R_a,*S*)-**4c**]	2.94	4.29
H^{γ}_{eq}···NHPhe [(S_a,*S*)-**4c**]	4.40	2.74
H^{γ}_{ax}···NHPhe [(S_a,*S*)-**4c**]	5.17	4.27
NHBz···NHPhe [(S_a,*S*)-**4c**]	2.98	4.36

[a] Distances are in Å.

Finally, we focused our attention on the side chain of these new axially chiral α,β-didehydroamino acids. Indeed, (R_a)- and (S_a)-4-methyl-, 4-phenyl- and (4-*tert*-butylcyclohexylidene)glycines can be regarded as alanine, phenylglycine

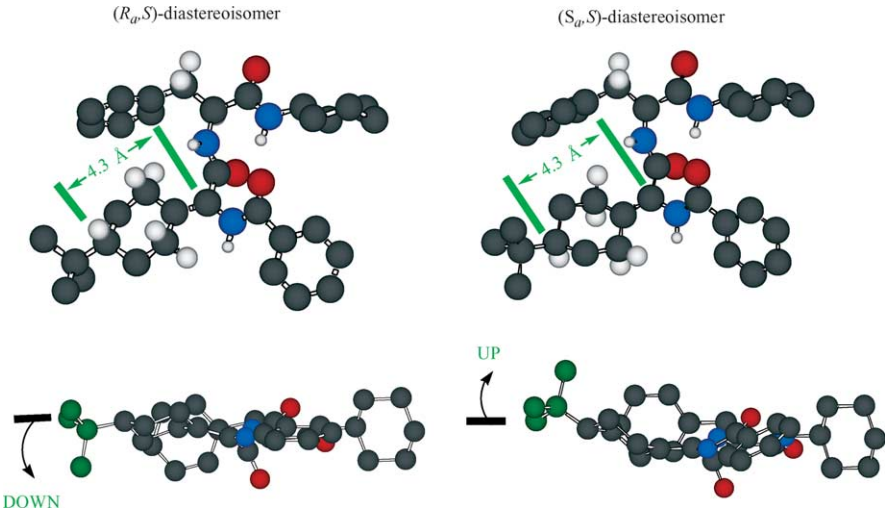


Figure 4. Spatial disposition of the side chain of (R_a)- and (S_a)-(4-*tert*-butylcyclohexylidene)glycine in compounds (R_a,S)-**4c** and (S_a,S)-**4c**.

and *tert*-leucine analogues of *R* and *S* configuration that contain conformationally locked spacers between the side chain of the amino acid and the backbone. In the model dipeptides (R_a,S)-**4a–c** and (S_a,S)-**4a–c**, which incorporate these axially chiral amino acids, the methyl, phenyl and *tert*-butyl groups are located 4.3 Å away from the peptide backbone and directed either down, (R_a,S) diastereoisomer, or up, (S_a,S) diastereoisomer, with respect to the middle plane of the β-turn. (Fig. 4).

3. Conclusions

Diastereomerically pure model dipeptides containing axially chiral α,β-didehydroamino acids at the ($i+1$) position can be conveniently obtained by applying the 'oxazolone method' developed by Obrecht et al.[15]

X-ray diffraction analyses of monocrystalline samples of compounds (R_a,S)-**4a** and (R_a,S)-**4c** revealed that in the crystal both compounds adopt a type II′ β-turn conformation with the two amino acids in the β-turn positions and a transannular hydrogen bond between the benzoyl C═O group and the cyclohexylamide NH group. NMR studies clearly indicate that this structure prevails in solution and reveals a similar behaviour for all synthesised compounds. It can therefore be concluded that axially chiral α,β-didehydroamino acids can accommodate in the ($i+1$) position of a β-turn in the heterochiral as well as in the homochiral dipeptides studied.

The introduction of axially chiral amino acids in model dipeptides allows the synthesis of conformationally restricted analogues in which the side chain of the amino acid is pulled out from the backbone peptide.

The development of synthetic methodologies to gain access to peptides that incorporate new axially chiral amino acids in different positions of the main chain is currently underway. In this context, the Wittig–Horner olefination has proven to be synthetically useful to obtain model dipeptides containing chiral α,β-didehydroamino acid moieties in the ($i+2$) position as diastereomeric mixtures.[22] Development of diastereoselective syntheses is underway and will be published in due course.

4. Experimental

4.1. General

All reagents were of analytical grade and were used as obtained from commercial sources. Most reactions were monitored by TLC. TLC was performed on precoated silica gel polyester plates and products were visualised using UV light (254 nm) and anisaldehyde/sulfuric acid/ethanol (2:1:100). Column chromatography was performed using silica gel (Kiesegel 60, 230–400 mesh). (*S*)-Phenylalanine cyclohexylamide (**3**) was prepared according to the previously described procedure.[15]

Melting points were determined in open capillaries and are uncorrected. FTIR spectra of liquids were recorded as thin films on NaCl plates and FTIR spectra of solids were recorded as nujol dispersions on NaCl plates, ν_{max} expressed in cm^{-1} is given for the main absorption bands and prominent peaks. Optical rotations were measured in a cell with a 10 cm path length at 25 °C, concentrations are given in g/100 mL. Elemental analyses were performed using a C, H, N, S elemental analyser. ^1H NMR and ^{13}C NMR spectra were acquired at 25 °C in $CDCl_3$ at 300 and 75 MHz, respectively. The chemical shifts (δ) are reported in parts per million and the coupling constants (J) in Hertz. The following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; bs, broad signal; bd, broad doublet; dd, doublet of doublets, ddd, doublet of doublet of doublets. The concentration of samples amounted to 10 mM for ^1H NMR, COSY spectra, and ^1H NOE experiments. No special precautions, such as degassing of the samples, were taken. In the NOE experiments Bruker standard microprogram NOEMULT was used and scans were recorded on applying 70 dB decoupling power, 8 s total irradiation time and 1 s relaxation delay. All two dimensional correlated spectroscopy (COSY) data consisted of 256 t_1 increments with a relaxation delay of 1 s. Shifted squared sine functions were applied to the data before transformation.

4.2. X-ray diffraction

The X-ray diffraction data were collected at room temperature on a four circle Siemens P4 diffractometer, using graphite-monochromated Mo Kα radiation (λ= 0.71073 Å). Structures were solved by direct methods using SIR92[23] and refinement was performed using SHELXL 97[24] by the full-matrix least-squares technique with anisotropic thermal factors for heavy atoms. Hydrogen atoms were calculated at idealised positions, and during refinement they were allowed to ride on their carrying atom with an isotropic thermal factor fixed to 1.2 times the U_{eq} value of the carrier atom (1.5 for the methyl protons).

Crystallographic data for the structures of compounds (R_a,*S*)-**4a** and (R_a,*S*)-**4c** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-210790 and 210789, respectively. Copies of the data can be obtained, free of charge, via http://www.ccdc.cam.uk/conts/retrieving.html or on application to CCDC, 12 Union Road, Cambridge CB2 1EZ. UK [fax: +44 9 1223 336033 or e-mail deposit@ccdc.cam.ac.uk].

4.3. General procedure for the synthesis of 4-(4-substituted-cyclohexylidene)-2-phenyl-1,3-oxazol-5(4*H*)ones (2a–c)

Ac_4Pb (2.22 g, 5 mmol) was added at room temperature to a mixture of the corresponding 4-substituted-cyclohexanone **1a–c** (30 mmol), hippuric acid (1.74 g, 10 mmol) and Ac_2O (3.06 g, 30 mmol). The reaction mixture was stirred for 5 h under reflux and then cooled to 0 °C, treated with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification of the residue by flash chromatography (eluting with diethyl ether/hexane 1/4) afforded the corresponding product **2a–c**.

4.3.1. 4-(4-Methylcyclohexylidene)-2-phenyl-1,3-oxazol-5(4*H*)one (2a). The above general procedure, starting from 4-methylcyclohexanone (**1a**) (3.36 g, 30 mmol), gave compound **2a** (1.56 g, 61%) as a white solid after column chromatography. Mp = 86 °C; IR absorption (nujol) 1782, 1744, 1659 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.94 (d, J=6.5 Hz, 3H), 1.12–1.27 (m, 2H), 1.68–1.75 (m, 1H), 1.93–2.02 (m, 2H), 2.13–2.29 (m, 2H), 3.45 (bd, J= 12.6 Hz, 1H), 3.85 (bd, J=13.8 Hz, 1H), 7.44–7.52 (m, 3H), 8.00–8.03 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 28.2, 31.4, 31.9, 35.8, 36.0, 126.1, 127.6, 128.7, 129.0, 132.3, 159.2, 161.1, 165.7. Elemental analysis calcd (%) for $C_{16}H_{17}NO_2$: C, 75.27; H, 6.71; N, 5.49; found: C, 75.56; H, 6.63; N, 5.57.

4.3.2. 4-(4-Phenylcyclohexylidene)-2-phenyl-1,3-oxazol-5(4*H*)one (2b). The above general procedure, starting from 4-phenylcyclohexanone (**1b**) (5.22 g, 30 mmol), gave compound **2b** (1.62 g, 51%) as a white solid after column chromatography. Mp = 128 °C; IR absorption (nujol) 1791, 1755, 1664 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 1.69–1.79 (m, 2H), 2.20–2.40 (m, 4H), 2.85–2.93 (m, 1H), 3.70 (bd, J=13.8 Hz, 1H), 4.10 (bd, J=13.6 Hz, 1H), 7.18–7.31 (m, 5H), 7.44–7.55 (m, 3H), 8.04–8.07 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.6, 31.7, 34.9, 35.0, 43.7, 126.0, 126.4, 126.7, 127.6, 128.5, 128.8, 129.5, 132.5, 145.4, 159.3, 159.6, 165.5. Elemental analysis calcd (%) for $C_{21}H_{19}NO_2$: C, 79.47; H, 6.03; N, 4.41; found: C, 79.52; H, 6.09; N, 4.35.

4.3.3. 4-(4-*tert*-Butylcyclohexylidene)-2-phenyl-1,3-oxazol-5(4*H*)one (2c). The above general procedure, starting from 4-*tert*-butylcyclohexanone (**1c**) (4.62 g, 30 mmol), gave compound **2c** (1.54 g, 52%) as a white solid after column chromatography. Mp = 125 °C; IR absorption (nujol) 1784, 1755, 1659 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (s, 9H), 1.18–1.42 (m, 3H), 2.00–2.22 (m, 4H), 3.55 (bd, J=13.2 Hz, 1H), 3.98 (bd, J=14.1 Hz, 1H), 7.40–7.48 (m, 3H), 7.98–8.02 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.5, 28.8, 28.9, 28.9, 32.0, 32.5, 47.5, 126.2, 127.6, 128.7, 128.8, 132.3, 159.3, 161.5, 165.7. Elemental analysis calcd (%) for $C_{19}H_{23}NO_2$: C, 76.74; H, 7.80; N, 4.71; found: C, 77.68; H, 7.71; N, 4.67.

4.4. General procedure for the synthesis of N^2-[(R_a)-N^1-benzoyl-(4-substituted-cyclohexylidene)glycyl]-(*S*)-phenylalanine cyclohexylamide [(R_a,*S*)-4a–c] and N^2-[(S_a)-N^1-benzoyl-(4-substituted-cyclohexylidene)glycyl]-(*S*)-phenylalanine cyclohexylamide [(S_a,*S*)-4a–c]

A mixture of the corresponding 5(4*H*)-oxazolone **2a–c** (1.5 mmol) and (*S*)-phenylalanine cyclohexylamide (**3**) (492 mg, 2 mmol) in *N*-methylpyrrolidin-2-one (8 mL) was stirred under argon for 24 h at 70 °C. The reaction mixture was cooled to room temperature and poured onto a mixture of ice (10 g), 1 N HCl (15 mL) and EtOAc (40 mL). The organic layer was washed with water and the combined aqueous layers extracted with EtOAc. The combined organic layers were washed with saturated brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification of the residue by flash chromatography, eluting first with Et_2O and then with EtOAc, afforded the corresponding peptide **4a–c** as an equimolecular mixture of diastereoisomers. Careful column chromatography, eluting with CH_2Cl_2/EtOAc 1/4, afforded analytically pure samples of the two diastereoisomers of compounds **4a–c**.

4.4.1. N^2-[(R_a)-N^1-Benzoyl-(4-methylcyclohexylidene) glycyl]-(*S*)-phenylalanine cyclohexylamide [(R_a,*S*)-4a]. The above general procedure, starting from 5(4*H*)-oxazolone **2a** (382 mg, 1.5 mmol), gave compound **4a** (691 mg, 92%) as an equimolecular mixture of diastereoisomers. Compound (R_a,*S*)-**4a** was eluted first upon additional column chromatography and isolated as a white solid. Mp = 195 °C (dec); $[\alpha]_D^{25} = -74.4$ (c 0.5, MeOH); IR absorption (nujol) 3292, 1650, 1635 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.85 (d, J=6.5 Hz, 3H), 1.00–1.94 (m, 17H), 2.09 (bd, J=14.4 Hz, 1H), 2.52 (bd, J=14.4 Hz, 1H), 3.09 (dd, J=14.3, 8.9 Hz, 1H), 3.39 (dd, J=14.3, 4.9 Hz, 1H), 3.69–3.82 (m, 1H), 4.82 (ddd, J=8.9, 8.5, 4.9 Hz, 1H), 6.05 (d, J=8.5 Hz, 1H), 7.17–7.22 (m, 6H), 7.28–7.36 (m, 2H), 7.40–7.48 (m, 1H), 7.66–7.71 (m, 2H), 8.26 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 25.2, 25.3, 25.7, 28.8, 29.1, 31.7, 32.6, 32.8, 34.5, 35.6, 37.5, 48.7, 54.6, 123.0, 126.9, 127.4, 128.3, 128.7, 129.1, 131.7, 132.3, 137.1, 139.7, 166.8, 167.2, 169.5. Elemental analysis

calcd (%) for $C_{31}H_{39}N_3O_3$: C, 74.22; H, 7.84; N, 8.38; found: C, 74.16; H, 7.79; N, 8.45.

4.4.2. N^2-[(S_a)-N^1-Benzoyl-(4-methylcyclohexylidene)-glycyl]-(*S*)-phenylalanine cyclohexylamide [(S_a,*S*)-4a]. Compound (S_a,*S*)-**4a** was eluted second upon additional column chromatography and isolated as a white solid. Mp = 245 °C (dec); $[\alpha]_D^{25} = -84.2$ (*c* 0.5, MeOH); IR absorption (nujol) 3303, 1656, 1631 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (d, *J* = 6.5 Hz, 3H), 1.00–1.97 (m, 17H), 2.09 (bd, *J* = 14.0 Hz, 1H), 2.49 (bd, *J* = 13.6 Hz, 1H), 3.12 (dd, *J* = 14.7, 8.9 Hz, 1H), 3.41 (dd, *J* = 14.7, 4.6 Hz, 1H), 3.70–3.85 (m, 1H), 4.89 (ddd, *J* = 8.9, 8.9, 4.6 Hz, 1H), 6.04 (d, *J* = 8.9 Hz, 1H), 7.17–7.28 (m, 7H), 7.33–7.41 (m, 1H), 7.58 (d, *J* = 8.1 Hz, 1H), 7.64–7.72 (m, 2H), 8.91 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.7, 25.3, 25.4, 25.7, 29.1, 29.3, 32.4, 32.6, 32.9, 35.0, 36.0, 37.3, 48.7, 54.6, 123.1, 127.0, 127.4, 128.1, 128.7, 129.0, 131.7, 132.1, 137.2, 138.3, 166.7, 167.8, 169.3. Elemental analysis calcd (%) for $C_{31}H_{39}N_3O_3$: C, 74.22; H, 7.84; N, 8.38; found: C, 74.21; H, 7.92; N, 8.42.

4.4.3. N^2-[(R_a)-N^1-Benzoyl-(4-phenylcyclohexylidene)-glycyl]-(*S*)-phenylalanine cyclohexylamide [(R_a,*S*)-4b]. The above general procedure, starting from 5(4*H*)-oxazolone **2b** (475 mg, 1.5 mmol), gave compound **4b** (709 mg, 84%) as an equimolecular mixture of diastereoisomers. Compound (R_a,*S*)-**4b** was eluted first upon additional column chromatography and isolated as a white solid. Mp = 201 °C (dec); $[\alpha]_D^{25} = -103.0$ (*c* 0.5, MeOH); IR absorption (nujol) 3297, 1637 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.00–2.04 (m, 17H), 2.32 (bd, *J* = 14.4 Hz, 1H), 2.60 (bd, *J* = 12.2 Hz, 1H), 3.11 (dd, *J* = 14.3, 8.6 Hz, 1H), 3.36 (dd, *J* = 14.3, 5.3 Hz, 1H), 3.72–3.84 (m, 1H), 4.82 (ddd, *J* = 8.7, 8.6, 5.3 Hz, 1H), 6.13 (d, *J* = 8.7 Hz, 1H), 7.09–7.32 (m, 11H), 7.36–7.44 (m, 2H), 7.46–7.54 (m, 1H), 7.73–7.76 (m, 2H), 7.91 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.2, 25.3, 25.7, 29.5, 29.7, 32.7, 32.8, 33.9, 34.5, 37.4, 43.5, 48.7, 54.4, 123.5, 126.3, 126.6, 127.0, 127.4, 128.4, 128.5, 128.7, 129.2, 131.9, 132.3, 136.9, 138.9, 145.6, 166.9, 167.0, 169.4. Elemental analysis calcd (%) for $C_{36}H_{41}N_3O_3$: C, 76.70; H, 7.33; N, 7.45; found: C, 76.83; H, 7.25; N, 7.38.

4.4.4. N^2-[(S_a)-N^1-Benzoyl-(4-phenylcyclohexylidene)-glycyl]-(*S*)-phenylalanine cyclohexylamide [(S_a,*S*)-4b]. Compound (S_a,*S*)-**4b** was eluted second upon additional column chromatography and isolated as a white solid. Mp = 130 °C (dec); $[\alpha]_D^{25} = -98.8$ (*c* 0.5, MeOH); IR absorption (nujol) 3315, 1637 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.20–2.00 (m, 16H), 2.27 (bd, *J* = 14.3 Hz, 1H), 2.57 (dt, *J* = 11.8, 2.9 Hz, 1H), 2.67 (bd, *J* = 14.4 Hz, 1H), 3.15 (dd, *J* = 14.7, 8.7 Hz, 1H), 3.43 (dd, *J* = 14.7, 4.9 Hz, 1H), 3.75–3.90 (m, 1H), 4.95 (ddd, *J* = 8.9, 8.7, 4.9 Hz, 1H), 6.11 (d, *J* = 8.9 Hz, 1H), 7.12–7.28 (m, 12H), 7.28–7.39 (m, 1H), 7.65 (d, *J* = 8.5 Hz, 1H), 7.68–7.72 (m, 2H), 9.20 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.3, 25.4, 25.7, 29.5, 29.7, 32.7, 32.9, 34.3, 35.0, 37.3, 44.3, 48.7, 54.7, 123.7, 126.5, 126.7, 127.0, 127.5, 128.1, 128.6, 128.8, 129.1, 131.7, 131.9, 137.1, 137.3, 166.8, 167.9, 169.2. Elemental analysis calcd (%) for $C_{36}H_{41}N_3O_3$: C, 76.70; H, 7.33; N, 7.45; found: C, 76.81; H, 7.39; N, 7.51.

4.4.5. N^2-[(R_a)-N^1-Benzoyl-(4-*tert*-butylcyclohexylidene)-glycyl]-(*S*)-phenylalanine cyclohexylamide [(R_a,*S*)-4c]. The above general procedure, starting from 5(4*H*)-oxazolone **2c** (445 mg, 1.5 mmol), gave compound **4c** (688 mg, 82%) as an equimolecular mixture of diastereoisomers. Compound (R_a,*S*)-**4c** was eluted first upon additional column chromatography and isolated as a white solid. Mp = 158 °C (dec); $[\alpha]_D^{25} = -72.4$ (*c* 0.5, MeOH); IR absorption (nujol) 3319, 1661, 1640 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.82 (s, 9H), 0.98–1.98 (m, 17H), 2.37 (bd, *J* = 13.3 Hz, 1H), 2.62 (bd, *J* = 14.0 Hz, 1H), 3.24 (d, *J* = 6.7 Hz, 2H), 3.66–3.84 (m, 1H), 4.80 (ddd, *J* = 8.7, 6.7, 6.7 Hz, 1H), 6.03 (d, *J* = 8.7 Hz, 1H), 7.15–7.27 (m, 6H), 7.31–7.39 (m, 2H), 7.42–7.50 (m, 1H), 7.67–7.73 (m, 2H), 8.00 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.1, 25.2, 25.7, 27.4, 27.5, 28.6, 29.7, 30.1, 32.4, 32.6, 32.7, 37.3, 47.4, 48.7, 54.3, 122.7, 127.0, 127.2, 128.4, 128.8, 129.4, 131.8, 132.4, 136.7, 140.5, 166.6, 166.8, 169.4. Elemental analysis calcd (%) for $C_{34}H_{45}N_3O_3$: C, 75.10; H, 8.34; N, 7.73; found: C, 75.22; H, 8.25; N, 7.83.

4.4.6. N^2-[(S_a)-N^1-Benzoyl-(4-*tert*-butylcyclohexylidene)-glycyl]-(*S*)-phenylalanine cyclohexylamide [(S_a,*S*)-4c]. Compound (S_a,*S*)-**4c** was eluted second upon additional column chromatography and isolated as a white solid. Mp = 129 °C (dec); $[\alpha]_D^{25} = -87.2$ (*c* 0.5, MeOH); IR absorption (nujol) 3298, 1634 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (s, 9H), 0.94–1.98 (m, 17H), 2.24 (bd, *J* = 13.9 Hz, 1H), 2.56 (bd, *J* = 13.3 Hz, 1H), 3.23 (dd, *J* = 14.3, 8.4 Hz, 1H), 3.34 (dd, *J* = 14.3, 4.9 Hz, 1H), 3.77–3.85 (m, 1H), 4.89 (ddd, *J* = 8.7, 8.4, 4.9 Hz, 1H), 6.02 (d, *J* = 8.7 Hz, 1H), 7.11–7.37 (m, 7H), 7.31–7.42 (m, 1H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.63–7.73 (m, 2H), 8.89 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.3, 25.4, 25.7, 27.5, 27.7, 28.7, 29.6, 29.9, 32.4, 32.7, 32.8, 37.2, 47.8, 48.7, 54.5, 122.7, 127.1, 127.4, 128.2, 128.8, 129.2, 131.7, 132.1, 137.0, 138.5, 166.8, 167.7, 169.3. Elemental analysis calcd (%) for $C_{34}H_{45}N_3O_3$: C, 75.10; H, 8.34; N, 7.73; found: C, 74.98; H, 8.28; N, 7.79].

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