Anthranilic Acid-containing Cyclic Tetrapeptides: At The Crossroads Of Conformational Rigidity And Synthetic Accessibility

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Anthranilic Acid-containing Cyclic Tetrapeptides: At The Crossroads Of Conformational Rigidity And Synthetic Accessibility

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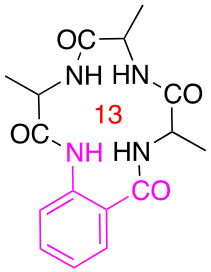
GRAPHICAL ABSTRACT

easier to prepare than cyclic tri- and tetra-peptides,
more rigid than 13-membered rings containing
other β-amino acids,
and adopts stereochemically-tunable
homogeneous conformations



ABSTRACT:

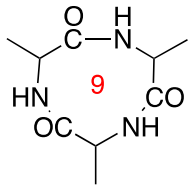
Each amino acid in a peptide contributes three atom units to main-chains, hence natural cyclic peptides can be 9, 12, 15, …. *ie* 3*n* membered-rings, where *n* is the number of amino acids. Cyclic peptides that are 9 or 12-membered ring compounds tend to be hard to prepare because of strain, while their one amino acid homologs (15-membered cyclic pentapeptides) are not conformationally homogeneous unless constrained by strategically placed proline or D-amino acid residues. We hypothesized that replacing one genetically encoded amino acid in a cyclic tetrapeptide with a *rigid* β-amino acid would render peptidomimetic designs that rest at a useful crossroads between synthetic accessibility and conformational rigidity. Thus this research explored non-proline containing 13-membered ring peptides **1** featuring one anthranilic acid (*Anth*) residue. Twelve cyclic peptides of this type were prepared, and in doing so the viability of both solution- and solid-phase methods was demonstrated. The library produced contained a complete set of four diastereoisomers of the sequence **1aaf** (*ie cyclo*-AlaAlaPhe*Anth*). Without exception, these four diastereoisomers each adopted one predominant conformation in solution; basically these conformations feature amide *N-H* vectors puckering above and below the equatorial plane, and approximately oriented their *N-H* atoms towards the polar axis. Moreover, the shapes of these conformers varied in a logical and predictable way (NOE, temperature coefficient, D/H exchange, circular dichroism). Comparisons were made of the side-chain orientations presented by compounds **1aaa** in solution with ideal secondary structures and protein-protein interaction interfaces. Various **1aaa** stereoisomers in solution present side-chains in similar orientations to regular and inverse γ-turns, and to the most common β-turns (types I and II). Consistent with this, compounds **1aaa** have a tendency to mimic various turns and bends at protein-protein interfaces. Finally, proteolytic- and hydrolytic stabilities of the compounds at different pHs indicate they are robust relative to related linear peptides, and rates of permeability through an artificial membrane indicate their structures are conducive to cell permeability.

INTRODUCTION

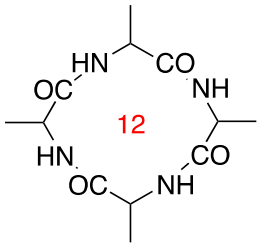
Cyclization of linear peptides increases their proteolytic stabilities and rigidities. In ideal cases these structures will adopt only *one* preferred conformation; if that occurs, less entropy will be surrendered on interaction with biomolecular receptors, increasing the free energies for the interactions. Observation of a strongly preferred conformation in solution also makes it probable that the molecule will bind to the receptor in a similar conformation, compared to other situations in which the compound exists in several solution conformations. Moreover, exclusion of competing conformational states reduces possibilities for off-target binding.

Inconveniently, cyclic peptides composed of the 20 genetically encoded amino acids miss a "sweet spot" ring size where conformational homogeneity is attained without compromising ease of syntheses. Thus, cyclic *tri*-[1-4] **A** and *tetra*-peptides **B**[5-11] are notoriously difficult to prepare because they are constrained in 9- and 12-membered ring conformations. Analogs of cyclic tetrapeptides, like the 12-membered ring system **C**, may be more easily prepared but another problem arises: *cis/trans* amide bond equilibria introduces conformational heterogeneity.[12] Cyclic *penta*peptides,[13-15] are easier to make than cyclic tri- or tetrapeptides[16] because their 15-membered rings are less strained, but they tend to equilibrate between conformers **D**$^{\mathbf{1–5}}$ containing β- and γ-turns. Certain states in the **D**$^{\mathbf{1–5}}$ equilibrium can be favored if one of the amino acids has a D-configuration, especially D-Pro,[17] but most cyclic pentapeptides and higher homologs overall do not tend to be rigid unless further constrained.[18,19]

a



A



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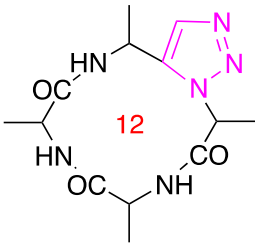
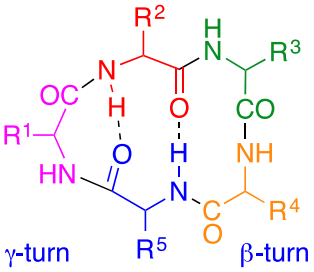


C

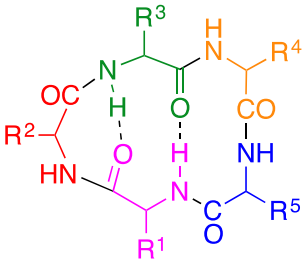
cyclic tri- and tetrapeptides: too difficult to prepare conveniently

accessible analog but conformationally heterogenous via amide cis/trans isomerism

b *cyclic pentapeptides: easy to make, but conformationally ambiguous*



D1



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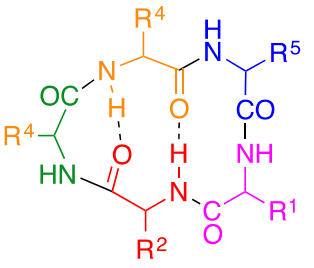

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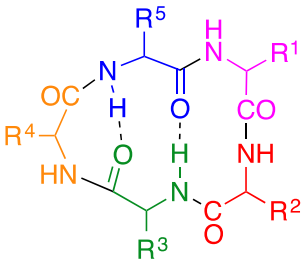
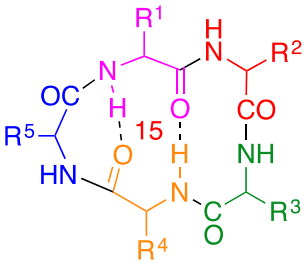


D4



D5

C *some known 13-membered ring cyclic peptidomimetics*

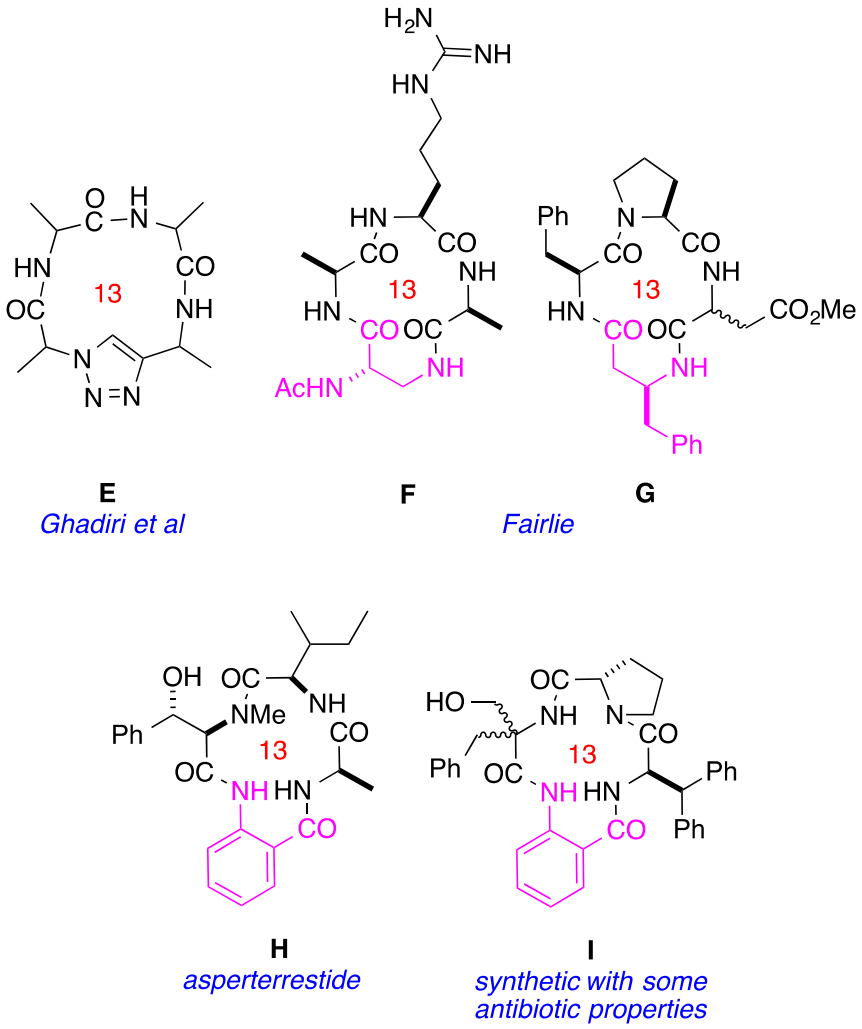


Figure 1. **a** 12-Membered ring peptidic systems (*eg* cyclic tetrapeptides) are highly constrained and their conformations may be complicated by *cis-trans* isomerism. **b** Cyclic pentapeptides tend to equilibrate between similar states containing both γ and β-turns, *ie* they tend to be conformationally heterogeneous. **c** 13-Membered rings may give only one preferred conformer (*eg* **E** - **G**) but this issue has not previously been studied for systems containing the *Anth* residue (*eg* for **H** and **I**).

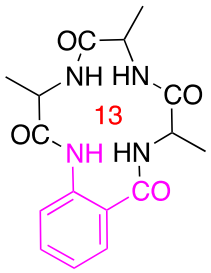
Based on the observations above, there should be favored ring sizes in peptidomimetic design where non-genetically encoded residues replace one amino acid to give conformationally rigid 13- or 14-membered rings. Ghadiri and co-workers, for instance,[20,21] have used copper-mediated azide-alkyne cycloadditions to give the 13-membered rings **E** which were conformationally rigid.[22] In other illustrative work, Fairlie *et al* substituted β-amino acids into

cyclic tetrapeptides and found some 13-membered ring systems, including **F**[23] and **G**[24], that could be prepared efficiently, and were conformationally rigid. However, that same work showed similar, but conformationally *heterogeneous*, 13-membered ring systems.[24]

Anthranilic acid is readily available and more rigid than most other β-amino acids. Several peptidic macrocycles containing anthranilic acid occur in Nature, most where this unit is one of five in a pentapeptide ring.[25-38] There are also numerous examples of similar hexapeptides and higher homologs incorporating anthranilic acid,[25,39-45] a few 10-membered tripeptide derivatives[46-48] and several cyclic systems containing the "*Anth*" residue and another non-encoded amino acid.[49-51] However, 13-membered ring systems containing this ubiquitous residue have been under-explored. Only one natural tetrapeptide **H** that features *Anth* in a 13-membered ring has been discovered,[52] and the only 13-membered cyclic peptide containing *Anth* that has been synthesized is compound **I**, prepared as part of a medicinal chemistry project.[53] To the best of our knowledge, neither **H** nor **I** have been studied in solution to determine their conformational biases.

We hypothesized compounds **1** could be prepared from readily available starting materials, and would be conformationally rigid. This paper describes how those compounds were made, and the conformational biases of one complete set of enantiomers in this series. In the event the conformations of these molecules were shown to correlate with their chiral amino acid stereochemistries in a logical, easily understood, way that is useful for predicting the preferred shapes of these rigid scaffolds.

do these cyclic peptidomimetics occupy the sweet spot that combines synthetic accessibility and conformational homogeneity?



1

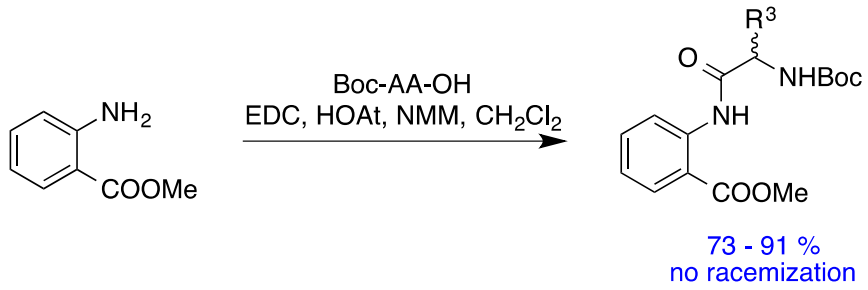
this work

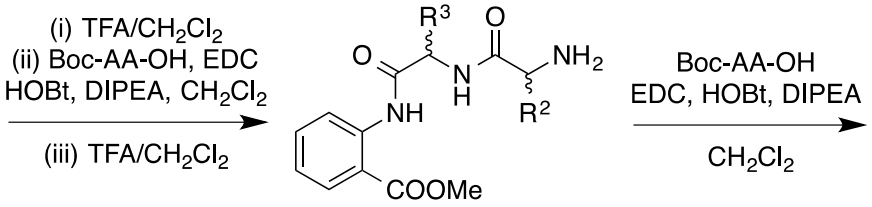
RESULTS AND DISCUSSION

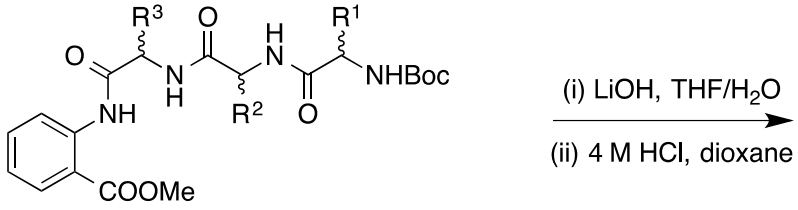
Syntheses Via Iterative Precipitations

Couplings to anthranilic acid are not facile because the aromatic amine is deactivated via resonance. However, Scheme 1 describes how solution-phase syntheses of several compounds were achieved using a large excess of *Anth* and a high concentration of all agents; if high concentrations were not used then epimerization was competitive with product formation. Use of a relatively weak base (*N*-methyl morpholine) and of the superior, though more expensive, coupling additive HOAt,[54] was also beneficial in this step.

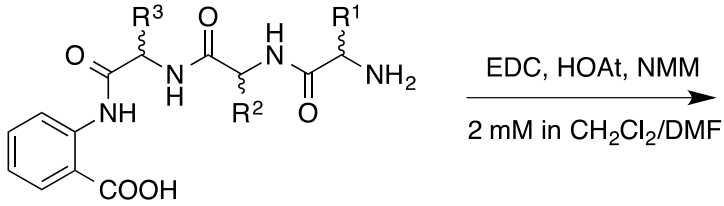
Early in this study we realized the physiochemical properties of peptides containing anthranilic acid facilitated their isolation. Thus, coupling three amino acids to the *Anth* unit gave products that precipitated from dichloromethane/hexanes with sufficient purities to use in the next steps. In fact, the only chromatography needed in the “Boc-approach” to the cyclic systems shown in Scheme 1 was to isolate the cyclized product. Serine and tyrosine residues in the compounds prepared were protected with benzyl groups. Glutamic acid side-chain protection was achieved using a *tert*-butyl ester, which withstands selective deprotection of the *N*-Boc functionality with 4M HCl.[55]

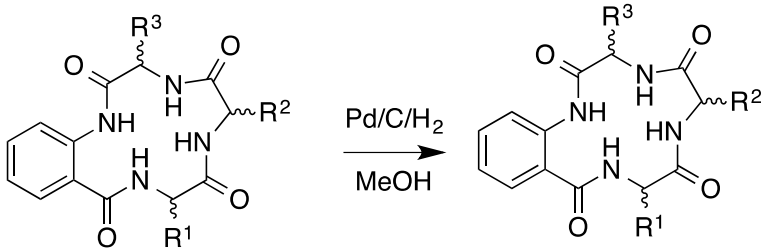






70 - 90 % over four steps





cyclo-LAla-LAla-LPhe-Anth, 41 %
cyclo-DAla-LAla-LPhe-Anth, 55 %
cyclo-LAla-DAla-LPhe-Anth, 48 %
cyclo-DAla-DAla-LPhe-Anth, 44 %
cyclo-DGlu'-DAla-LPhe-Anth, 35 %
cyclo-LVal-LSer'-LTyr'-Anth, 45 %
cyclo-DTyr'-DSer'-DVal-Anth, 31 %

cyclo-LVal-LSer-LTyr-Anth, 87 %

Scheme 1. *Boc approach* to products **1**.

Scheme S2 (supporting) shows a similar solution-phase approach to the same types of products but using Cbz protected amino acids and *N*α-deprotection via hydrogenolysis. In one of these syntheses the first amino acid added was H-Glu(O^{t}Bu) where successive deprotection under acidic conditions, as in Scheme 1, might have eroded the yield of this product, but *N*-deprotection via hydrogenolysis circumvented this potential problem. A complementary *solid phase* Fmoc-approach was also established (Scheme S3, supporting), based on 2-chloro-trityl polystyrene resin and involves cleavage from the support then cyclization in the final step.

Conformational Analyses

One goal in this study was to elucidate the intrinsic conformational biases of the stereoisomeric scaffolds **1** with minimal perturbation from side-chain interactions. The ideal system to study might have been the simple peptide **1aaa** (or *cyclo*-AlaAlaAla*Anth*). However, there was insufficient dispersion of the ^{1}H NMR peaks in that particular compound to facilitate convenient conformational analyses. Consequently, we decided to study one enantiomeric series of the compounds **1aaf** (or *cyclo*-AlaAlaPhe*Anth*).

One-dimensional ^{1}H NMR of the **1aaf** series all showed sharp, resolved peaks for the side-chains and for the amide protons. All the amino acid amide *N*<u>*H*</u> resonances were split into doublets via coupling to the *C*<u>*H*</u> protons (see below, Table 1). These observations imply that the compounds do not equilibrate between different conformations on the NMR time-scale, and that in solution each exists predominantly in one form. Temperature coefficient data[56,57] and rates of H/D exchange[58,59] were also measured. Neither set of data were particularly informative, except that they indicate there are no "*endo*-cyclic" *H*-bonds, consistent with the conformations deduced from NMR and calculations that are described immediately below.

NOE data were collected for all the compounds, and no *cis*-amide bonds were present (Cα*H* – Cα*H* cross-peaks absent).

Two methods were used to deduce the predominant conformations of compounds **1** in polar solvents. First, the molecules **1aaa** were simulated in a medium of dielectric 46.7 representing DMSO (and 80 representing water, see supporting) using the quenched molecular dynamics (QMD) technique.[60,61] QMD simulations are valuable because they are not bias by NOE data which over-represents some conformations because the NOE effect depends on $1/r^6$ distance relationships.[62] Moreover, QMD thoroughly explores possible local minima in a Boltzmann equilibrium. Second, and independently, NOE restraints *were* applied in a Macromodel[63] simulation of molecules **1aaf** in dielectric 46.7.

One conformational cluster (maximum RMSD of the Cα – Cβ coordinates 0.5 Å) arose from the QMD simulations of each of the **1aaa** stereoisomers, Figure 2a (QMD simulated scaffold conformations are shown in black throughout this paper). The fact that >1000 conformers (all below 3 kcal•mol^{-1} of the lowest energy one identified) *all* converged to one cluster emphatically indicates conformational rigidity. Comparison of the QMD-generated structures with the NOE data showed they are consistent. Similarly, the MacroModel simulations *with NMR constraints* also gave one predominant conformation for each **1aaf** stereoisomer, Figure 2b (MacroModel simulated scaffold conformations involving NOE constraints are grey throughout). None of the $^3J_{NH\text{-}\alpha}$ coupling constants were above 9 Hz (see Table 1 below); only couplings >9 Hz are indicative of unambiguous calculated dihedral angles, hence none were used as constraints in the MacroModel calculations.

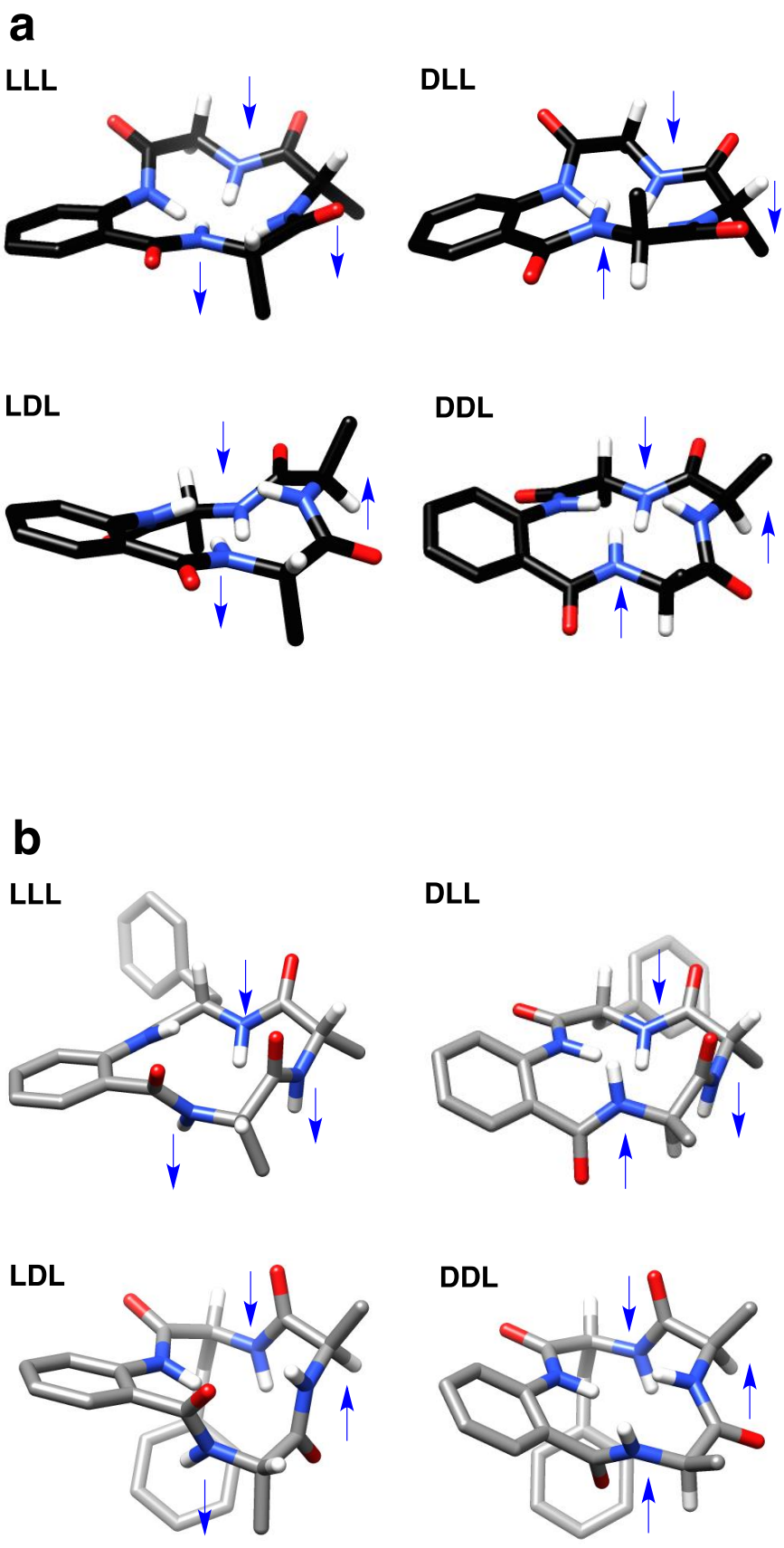


Figure 2. Conformations simulated by: **a** QMD calculations for **1aaa** stereomers *without* NOE constraints; and, **b** MacroModel for **1aaf** stereomers with NOE constraints. The blue arrows

approximate the orientation of the NH bond vectors to either pointing up or below the ring system.

Overlays of the conformations generated using the approaches described above, *ie* without and with NOE constraints, showed close agreement (RMSD 0.18 – 0.34 Å for the Cα – Cβ coordinates; Figure 3). Moreover, even though the fit of these overlays was based on Cα – Cβ vectors, it is clear that the ring structures also are very similar.

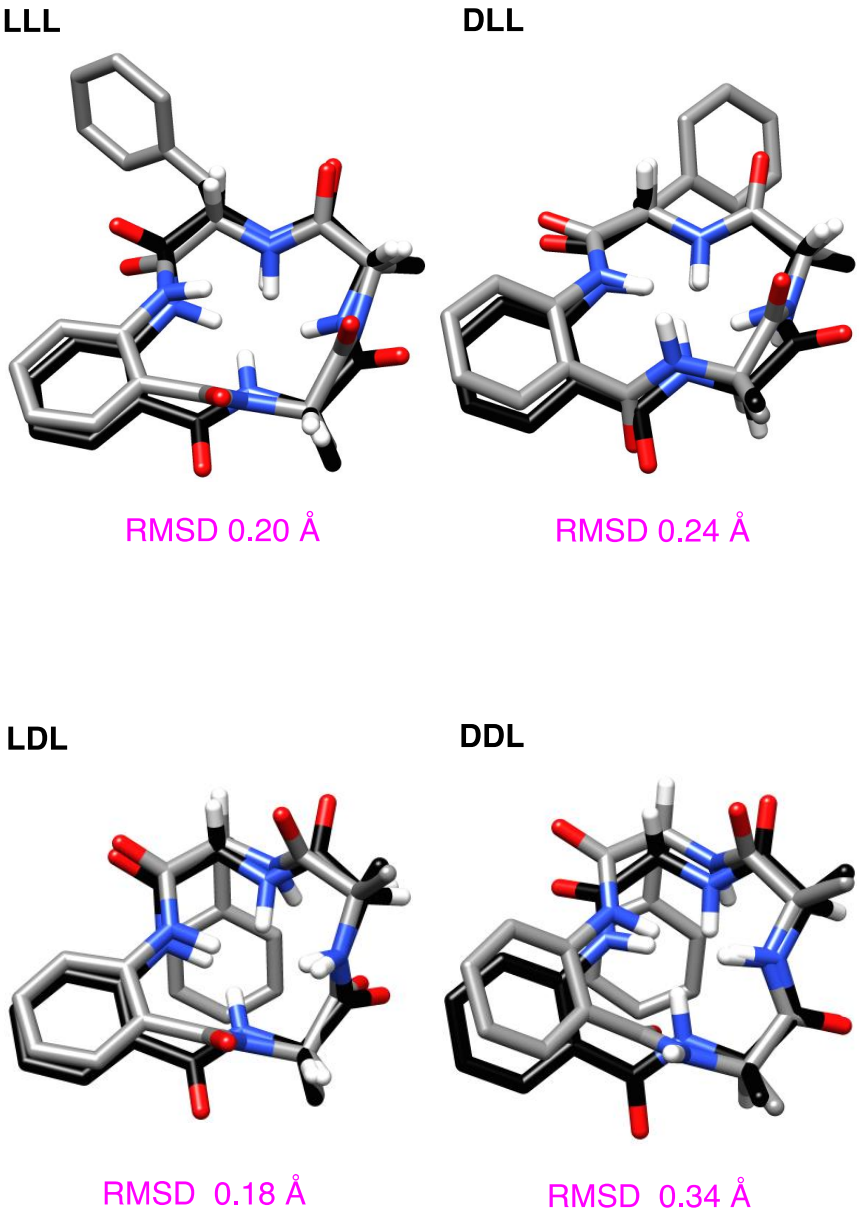


Figure 3. Overlays of **1** simulated structures without (black, **1aaa**) and with (grey, **1aaf**) NOE constraints are within 0.18 – 0.34 Å based on 6 coordinates.

Comparisons of favored conformations for stereomers of **1** revealed a simple correlation. When drawn with the ring on the equator, and the *Anth* residue on the West, then L-amino acids point their *N-H* vectors South, while *N-H* vectors for D-amino acids are North-oriented, *irrespective of the other amino acid stereochemistries*. Thus the cyclic 13-membered ring scaffold is constrained to one highly predictable conformation per stereoisomer. This observation held for all the compounds prepared in this study based on similarities in NOE cross-peaks, *ie* for *cyclo*-DGlu'-DAla-LPhe-Anth, *cyclo*-LVal-LSer'-LTyr'-Anth, *cyclo*-DTyr'-DSer'-DVal-Anth, *cyclo*-LVal-LSer-LTyr-Anth, *cyclo*-LPhe-DAla-LPhe-Anth, *cyclo*-DPhe-LAla-LGlu'-Anth, *cyclo*-DPhe-LAla-LGlu-Anth (see supporting); those observations imply the conformations are governed by the scaffold and the side-chain variables are less significant.

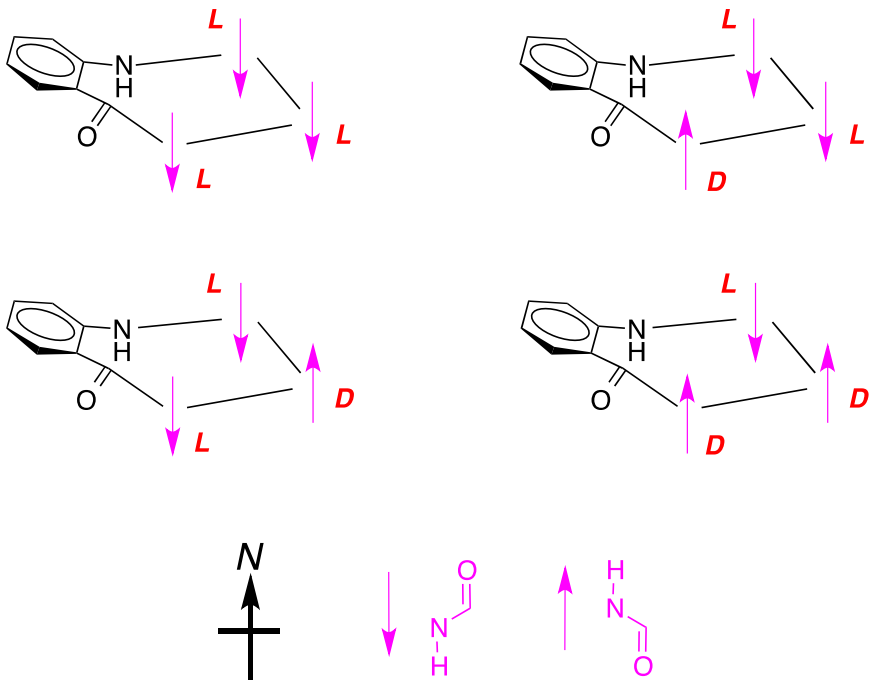


Figure 4. "North south orientations" of the amide *N-H* vectors in stereomers of **1** correlate with the amino acid configurations. L-Amino acid *N-H* vectors point South, and while the D-isomers give North-aligned local *N-H* vector orientations, when drawn in the orientations shown.

Table 1 compares N*H*-Cα*H* coupling constants directly from NMR spectra with those calculated from the MacroModel simulations involving NOE constraints. In some cases the coupling was obscured, and in several cases the true *J*-values were marginally (by 1.2 Hz at most) outside the calculated range, but the rest were consistent with the values inferred from simulations.

Table 1. Comparison of experimentally observed $^3J_{NH\text{-}\alpha}$ coupling constants with those calculated using the NMR constrained structures simulated in Figure 2b.

	Ala(R^1)		Ala(R^2)		Phe (R^3)	
	exp'l [a]	calc.[b]	exp'l [a]	calc.b	exp'l [a]	calc.[b]
LLL	5.0	6.2-6.7	-	7.8-8.3	7.0	6.5-7.8
DLL	7.7	7.6-8.2	8.9	6.6-7.9	7.9	8.0-9.0
LDL	7.2	6.8-8.0	8.8	7.9-8.9	7.7	6.9-8.1
DDL	5.2	5.6-6.3	-	6.6-7.1	5.6	6.6-7.1

[a] Directly from NMR spectra. [b] Based on the structures simulated from the NOE data, and calculated using the Poulson form of the Karplus equation.[64]

Circular dichroism (CD) data for the **1aaf** stereomers are shown in Figure 5 (solid lines). Unsurprisingly, significantly greater molar elipticities were observed for these compounds compared with related linear peptides (dotted lines), indicative of more conformational ordering in the cyclic systems. Moreover, there are logical trends in the data. For instance, the LLL-**1aaf** stereomer (red line) has negative maxima at *ca* 195 and 215, and a maximum at *ca* 230 nm. Substitution of two L-amino acids in LLL-**1aaf** giving DDL-**1aaf** is accompanied by near complete inversion of the CD maxima and minima. The two possible "intermediate" diastereomers, *ie* DLL-**1aaf** and LDL-**1aaf**, in which only one L-amino acid of LLL-**1aaf** is replaced, show shallower peak intensities. The CD spectrum of DLL-**1aaf** (green) is more closely related to that for LLL-**1aaf** (red) than it is to DDL-**1aaf** (purple), whereas for LDL-**1aaf** (blue) the inverse is true; this implies the amino acid opposite the *Anth* residue in the cyclic scaffold has a more profound effect on the molar elipticity.

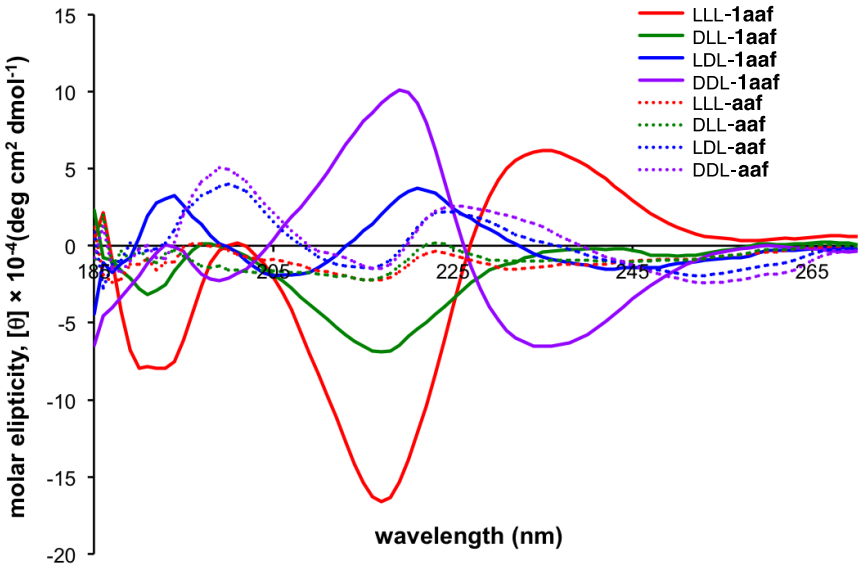


Figure 5. CD spectra of compounds **1aaf** (solid lines) and closely related linear peptides (dashed lines).

Comparisons Of The Anth-cyclic Peptidomimetics With Peptide And Protein Structures

Exploring key orientations on secondary structures (EKOS)[65] facilitates comparison of all the favored QMD simulated conformers with all the common ideal secondary structures based on Cα – Cβ coordinates. For **1aaf** there was only one preferred conformer cluster for each stereomer. Not surprisingly, then, most stereomers matched on only one secondary structure, or none at all, *ie* they are not universal peptidomimetics.[66,67] One apparent exception was LLL-**1aaf** which gave an acceptable fit on γ- and type II β-turn conformations (Figure 6); however, γ- and type II β-turn conformations have similar side-chain orientations that overlay well on each other (supporting Figure S1b). An inverse γ-turn matched reasonably well with LDL-**1aaf**, and DLL-**1aaf** overlaid closely with a type I β-turn.

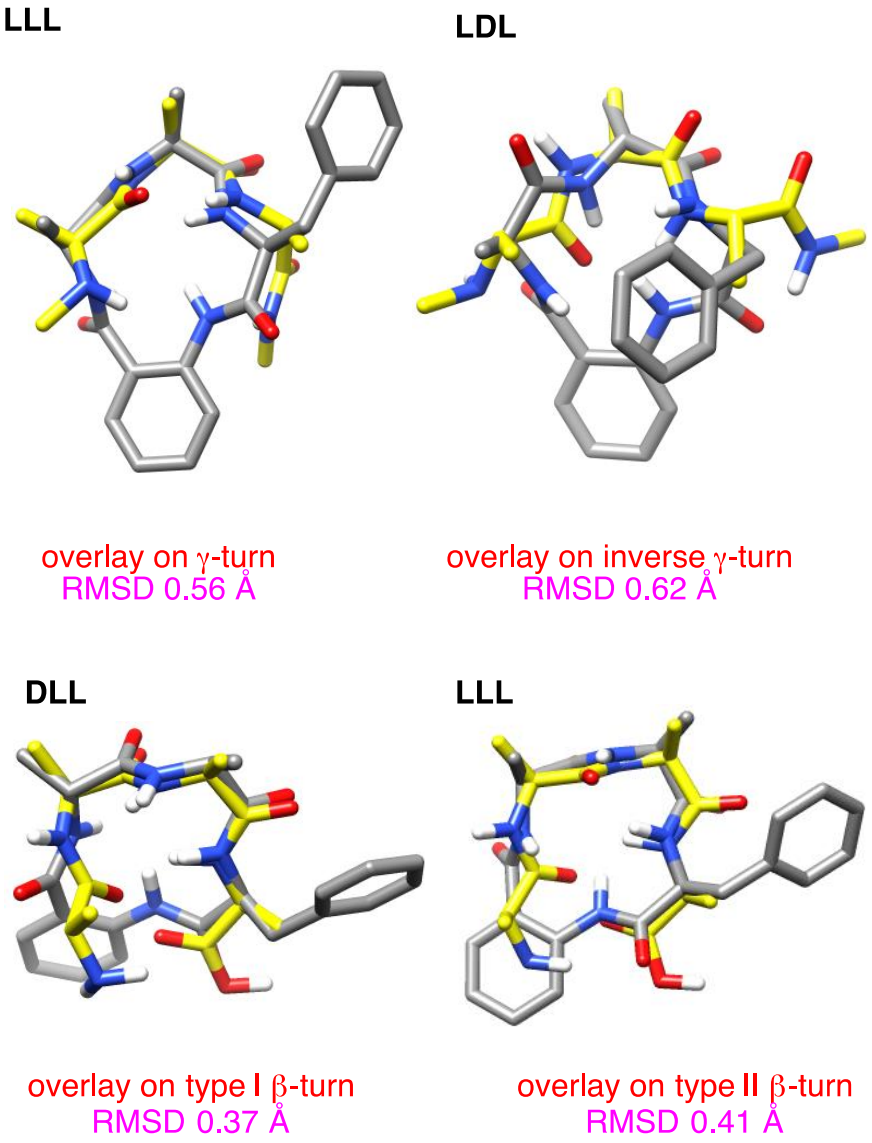
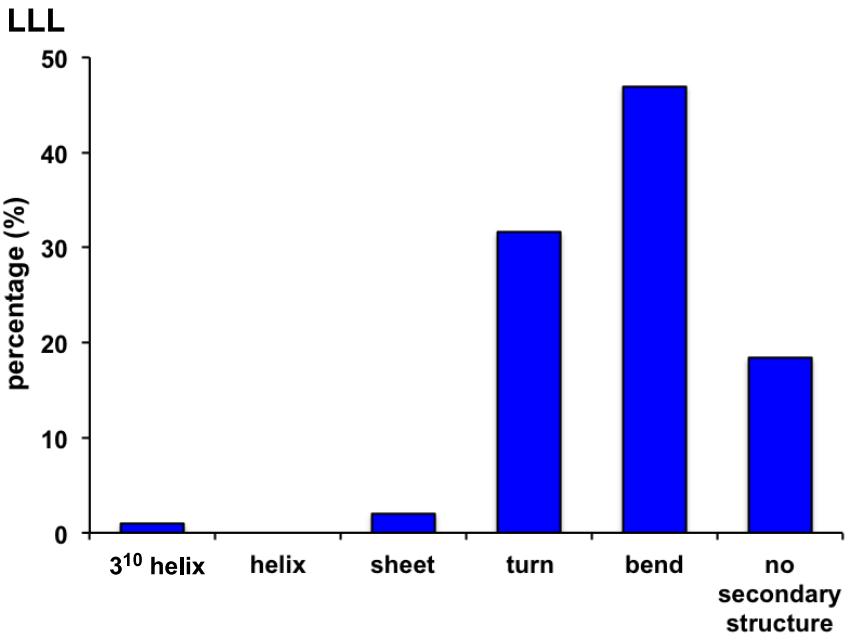


Figure 6. Preferred conformations of select stereoisomers of **1aaf** overlaid on γ-, inverse γ-, type I β-, and type II β-turns. RMSD values indicated are for overlay of the side-chain Cα – Cβ vectors.

Whereas, Figure 6 depicts preferred overlays of select **1aaf** stereoisomers on ideal secondary structures, the EKO routine[68] facilitates matching QMD generated structures of **1aaa** with crystallized protein-protein interaction interfaces based on Cα – Cβ coordinates. Thus, preferred conformations of each stereomer were compared with around 160,000 protein-protein interfaces, and each match of RMSD <0.3 Å was analyzed in terms of what secondary structure type at the interface was implicated in the overlay. There were between 106 - 258

matches of <0.3 Å RMSD for each stereomer. The term “no secondary structure” is used here to describe situations in which the region overlaid did not contain any discernable secondary structure. “Turns” refers to a turn of any type (α, β, γ, δ) that has appropriate intrachain hydrogen bonds, while loops and turns without any intra-ring *H*-bonding interactions are classified as “bends”. Figure 7 shows the distribution of overlays within those categories and some other secondary structures. Thus the preferred **1aaa** conformations are strongly bias towards turns and bends, consistent with the EKOS study presented in Figure 6. Full data for all the **1aaa** stereomers are given in the supporting (Figure S2).



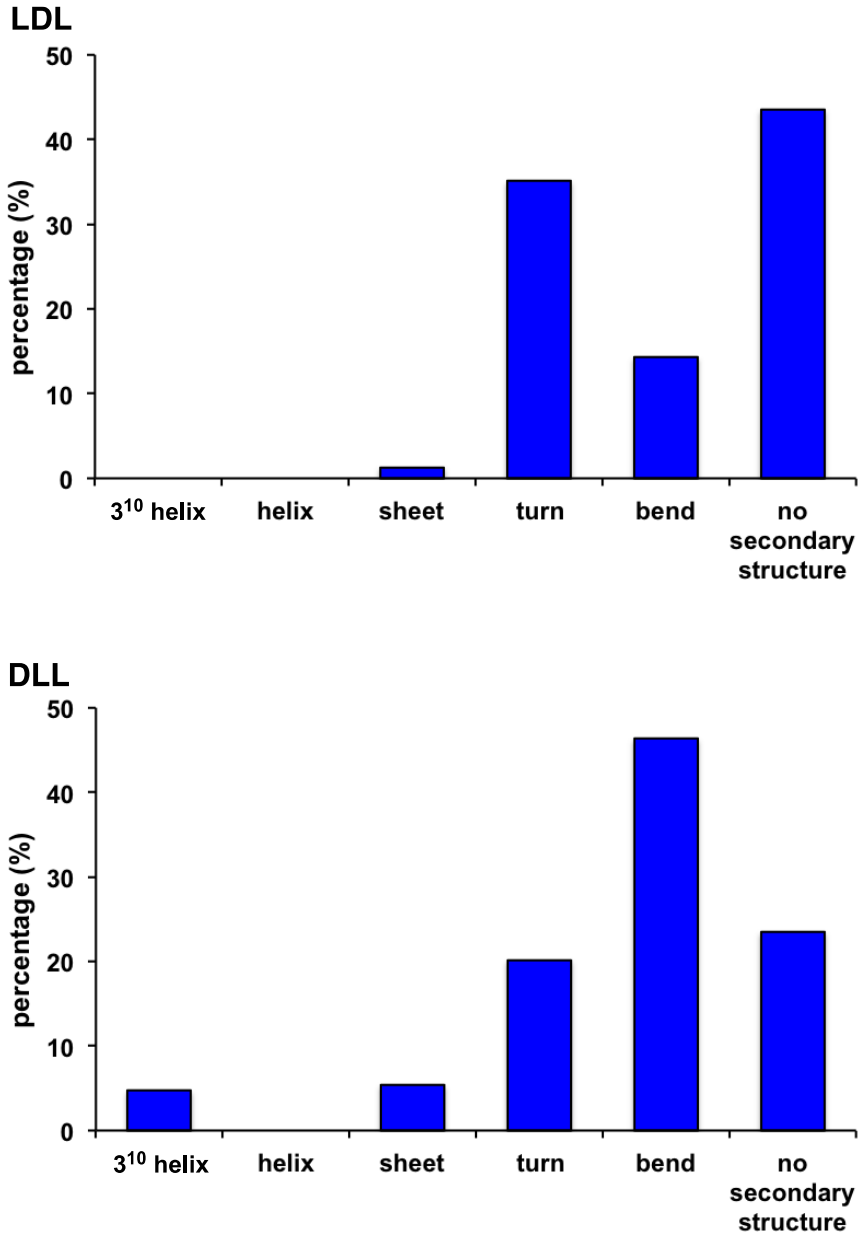
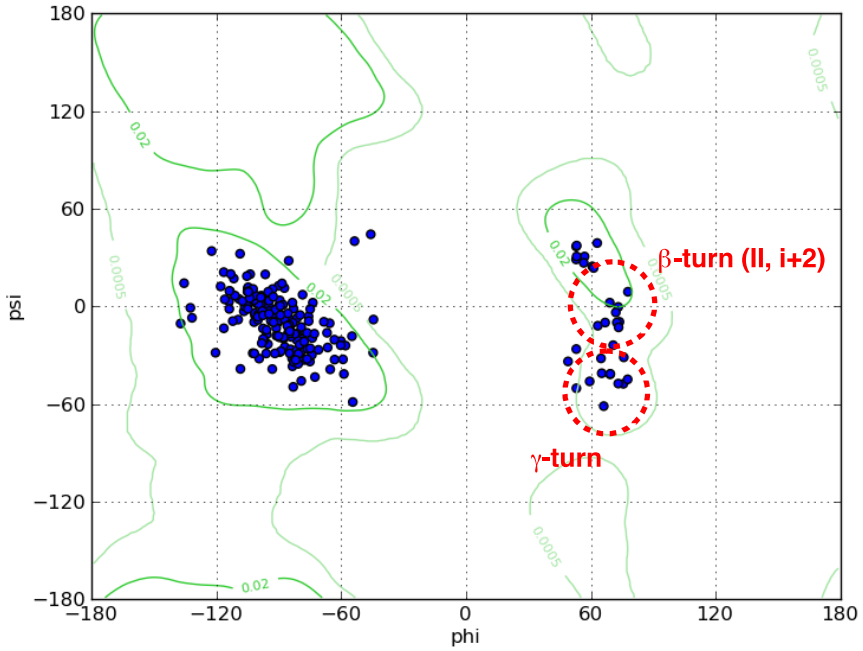


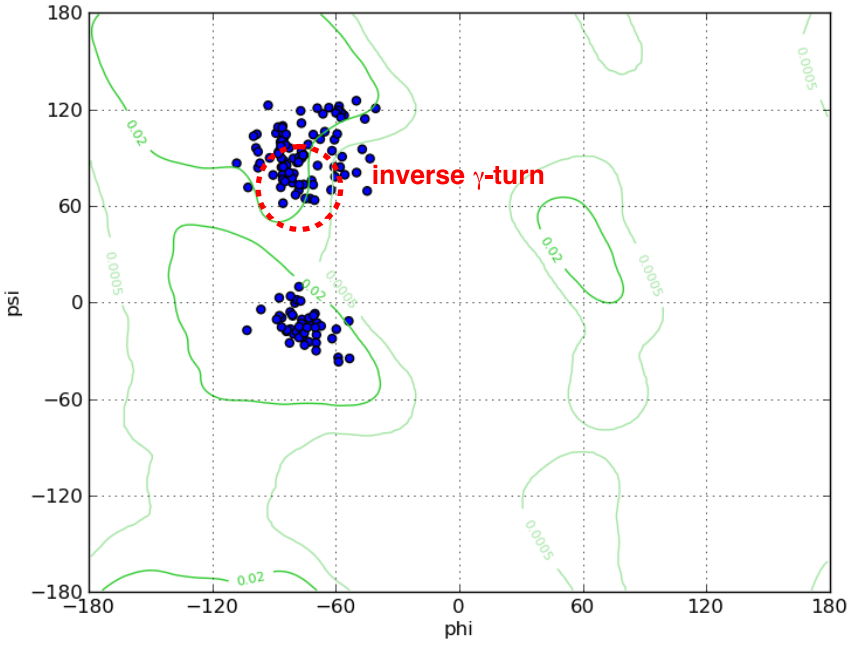
Figure 7. Distribution of best overlays on PPI interface segments with respect to secondary structure for the stereomers featured in Figure 6.

Finer detail of the secondary structure types that were overlaid in the EKO analysis of PPIs in the PDB is indicated by ϕ,ψ-angles, indicative of the type of secondary structure implicated. Figure 8 shows select data for the stereomers featured in Figure 6, and Figure S3 shows the complete data set. Thus, the number of occurrences where LLL-**1aaa**, LDL-**1aaa**, and DLL-**1aaa** overlaid closely with γ-turns, type I β-turns, inverse γ- and type II β-turn conformations are consistent with the favored conformations predicted in Figure 6.

LLL


β-turn (II, i+2)
γ-turn
psi
phi
180
120
60
0
−60
−120
−180
−180
−120
−60
0
60
120
180
0.02
0.0005

LDL


inverse γ-turn
psi
phi
180
120
60
0
−60
−120
−180
−180
−120
−60
0
60
120
180
0.02
0.0005

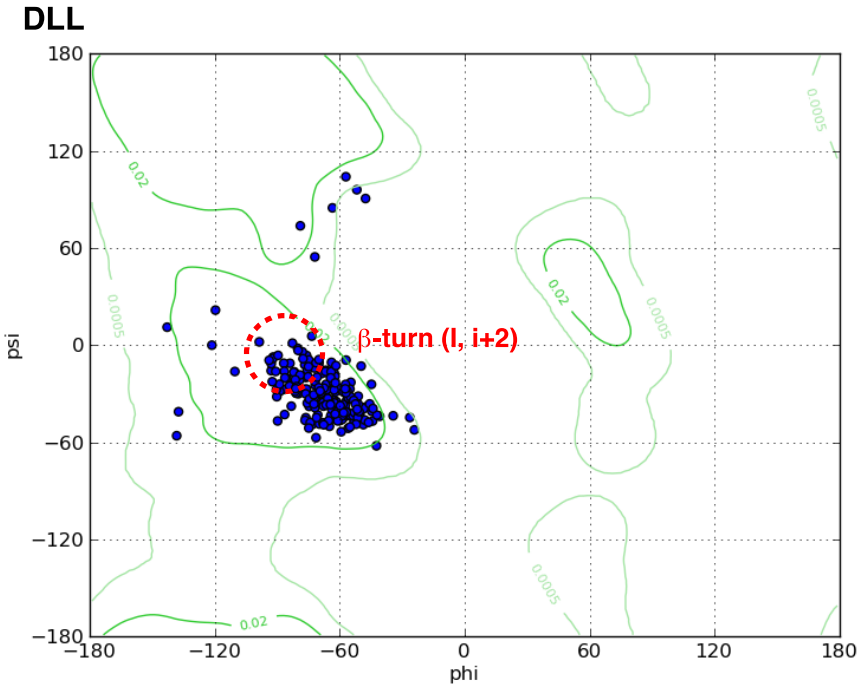


Figure 8. Each dot on these plots is associated with a ϕ,ψ-bond vector of a protein interface region that overlaid closely with a preferred conformer of the **1aaa** stereomer indicated.

Physiochemical Properties

Table 2 showshow HPLC was used to monitor the stability of LLL-**1aaf** under aqueous conditions of pH 2,7, and 12, and, in separate experiments, the mixture of proteases called *Pronase*[69] that is used to extensively hydrolyze proteins in proteomics studies (any amide between two hydrophobic residues could be cleaved by this enzyme mixture). Under neutral or acidic conditions, and in the presence of Pronase, LLL-**1aaf** did not any significant cleavage. Only some cleavage was observed after extended expose to the aqueous pH12 conditions.

Table 2. Stabilities of LLL-**1aaf** under different pH conditions

	conditions	$t_{1/2}$ / h
pH stability of LLL-**1aaf**	pH 2.0[a]	>500
	pH 12.0[b]	240
	pH 7.4[c]	>500
LLL-**1aaf**	Pronase[c]	no cleavage after 12 h
linear LLL-**aaf**	Pronase[c]	1.5

[a] 10 mM HCl, [b] 10 mM NaOH, [c] PBS buffer. 20 % MeOH was added in all cases to increase solubilities.

Predictions Of Cell Permeability

Figure 9 shows data calculated (QikProp)[70,71] for the polar surface area (PSA) and cell permeability of LLL-**1aaf**. The implications of this data are that LLL-**1aaf** has less PSA and a higher tendency to permeate into cells than closely related linear peptide controls. Polar surface areas <140 Å^2 are generally preferable for cell permeability.[72] Similarly, compounds with predicted PCaco-2 permeability rates >20 nm/s are usually cell permeable; data calculated for LLL-**1aaf** exceeds both expectations, and predict cell permeability.

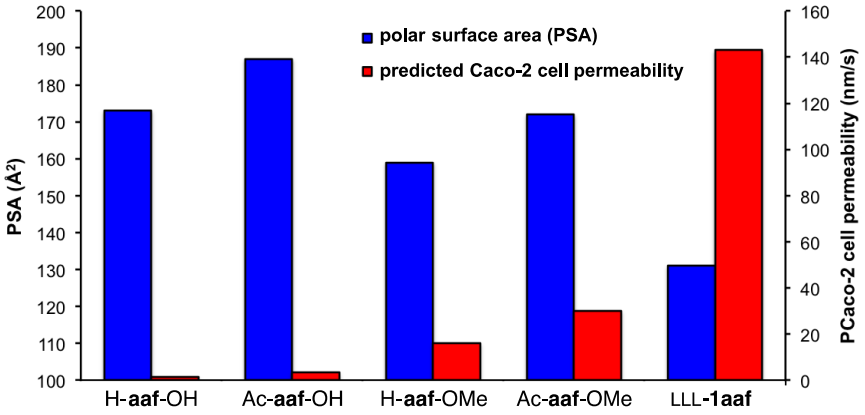


Figure 9. Comparison of PSA (blue) and predicted Caco-2 cell permeabilities (red) for linear peptides based on **aaf** and a featured cyclic molecule **1aaf**.

The parallel artificial membrane permeability assay (PAMPA)[73-77] was used to obtain experimental data to compare with QikProp calculations (Table 3). In PAMPA assays, a polyvinyldifluoroethene membrane is coated with mixture of a lipophilic hydrocarbon (here dodecane) and lipids, then the rate of diffusion of test compounds from donor to acceptor wells are measured over a period of time to mimic passive diffusion into cells. There is no uniformly accepted cut-off value in PAMPA assays above which the compounds are considered to be cell permeable, mainly because there is some variance in the membrane composition used. However, the following illustrative data for cell permeable pharmaceuticals have been obtained by others using exactly the method described here[78] (P_{app} in 10^6 • cm/s): Testosterone (13), Propranolol (10), Warfarin (1.0), Furosemide (0.16), Methotrexate (0.016). These data may serve as a reference for the data collected and shown in Table 3. That data suggests compounds based on the **aaf** core will tend to be cell permeable. Membrane permeability was reduced for the compounds with more polar side-chains, **vsy,** and for the linear control, LLL-H-**aaf**-OH as expected. This assertion is supported by another study that suggests P_{app} of around 1 x 10^6 • cm/s correlate with cell permeability.[79]

Others have noted[79] that data from PAMPA assays tends to be less than those from Caco2 assays, so the difference in the absolute values from QikProp (calculated Caco2 rates) and PAMPA are unsurprising. Moreover, there is a reasonable correlation between the relative rates in both sets of data.

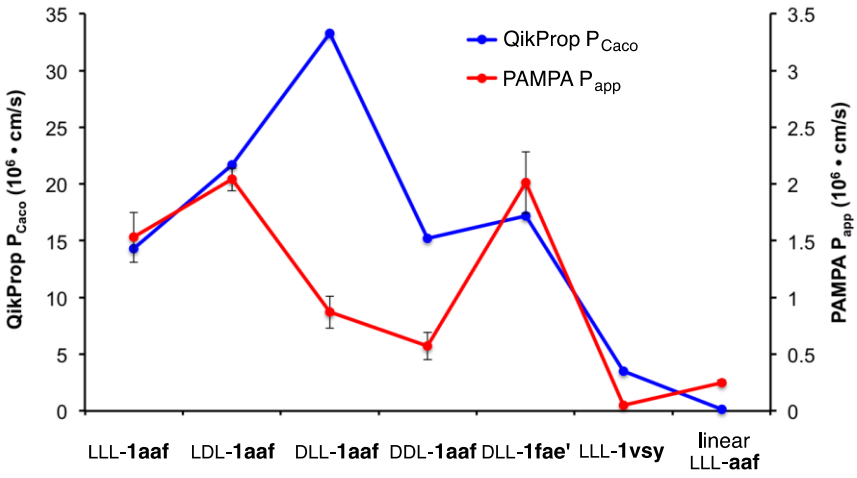


Figure 10. Calculated cell permeability rates from QikProp (blue), and experimental data from PAMPA assays (red).

CONCLUSION

The *Anth* containing peptides featured in this study have a greater tendency towards conformational homogeneity than systems based on other β-amino acids related to **F** and **G**.[24] In view of this, and other observations outlined in the introduction, we suggest 13-membered systems *based on the Anth residue* are at a favorable point on the crossroads between ease of synthesis and conformational rigidity.

Predictability of conformational preferences for a set of cyclic peptidomimetics is an attractive feature of the systems studied here. It is possible that different side-chains to the ones in **1aaf** could perturb the predicted conformations, and this study does not encompass the special case of systems that contain Pro- or Gly-. Nevertheless, the data above clearly indicates strong intrinsic conformational biases of the scaffolds based on other amino acids. Moreover, stereochemistries of the scaffolds can be manipulated so that they orient their side-chains in ways that resemble the same orientation for regular and inverse γ-turns, and for the two most common β-turns (types I and II). Consistent with this, conformations of **1aaa** stereomers have

a pronounced tendency to orient side-chains in ways that some turns and bends do at PPI interfaces.

Cyclic peptides containing only secondary amide bonds do not tend to be cell permeable.[80] In fact, making cyclic peptides cell permeable by inclusion of *N*-methyl groups is an area of interest in the contemporary literature.[81-83] Molecules **1** show promise as cell permeable molecules, even though they do not have tertiary amide *N*-methyl groups. We suggest this is because the favored conformers of **1** in solution orient their *N-H* vectors towards a central point, *ie* relatively insulated from the medium around the mimic.[84]

Overall, while tens of naturally occurring cyclic peptides containing *Anth* have been discovered, the potential of this simple residue in designs of conformationally rigid protein interface mimics has hitherto been under-appreciated.

ASSOCIATED CONTENT

Electronic Supplementary Information (ESI) available: experimental procedures and spectroscopic data for all compounds; H/D exchange experiments and temperature coefficient measurements; procedures and results for conformational modeling with QMD and MacroModel, results of the overlays on turn structures, data of protein database mining and the procedure for QikProp calculations; procedures for stability studies and the PAMPA assay.

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Notes

The authors declare no competing financial interests.

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