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Synthesis and Solid-state Conformations of 6*S*,8a*R/S*-6-Alkyl-3,3-dimethyltetrahydrooxazolo[3,4-*a*]pyrazine-5,8-diones (*Pseudo* proline Diketopiperazines)

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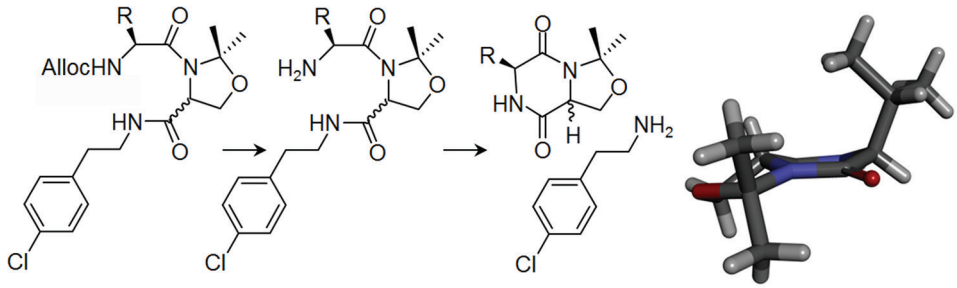
(Dedicated to Prof. S.P. Singh on his 80th Birthday)

ABSTRACT Peptide-like self-immolative molecular clips are required for release of active drugs from prodrugs by endopeptidases, generating diketopiperazines (DKPs) as by-products. Two diastereomeric series of *cyclo*-L-aminoacyl-*R*/*S*-dimethyloxazolidine carboxylate DKPs, derived from L-Ala, L-Leu and L Val, were synthesised. The oxazolidines were constructed by acid-catalysed condensation of N-protected L-Aaa-L/D-SerOMe dipeptides with $Me_2C(OMe)_2$, providing the protected 2,2-dimethyloxazolidine-carboxylates (Dmo) dipeptides. Deprotection exposed the primary amines, which cyclised rapidly to give the desired DKPs. The conformations were studied by nuclear magnetic resonance in $CDCl_3$ solution and, for the L,*R* series, by X-ray crystallography. The L,*S* series had the DKP in a boat conformation with the oxazolidine in a half-chair; the L,*R* series had the DKP in a flattened conformation with the oxazolidine in an alternative half-chair. Early kinetic studies have shown that L,*R*-Dmo dipeptide amides cyclised more rapidly than L,*S*-diastereoisomers and that the rate ofcyclisation in the L,*R* series depends inversely on steric bulk at the α-carbon. *Cyclo*-L-Leu-*R*-Dmo was non-toxic toward human HT29 cells.



KEY WORDS Diketopiperazine, Conformation, Prodrug, *Pseudo*proline, 2,2-dimethyloxazolidine-carboxylates.

INTRODUCTION

As part of our continuing development of prodrug systems,[1-6] we have studied molecular clips which could act as self-immolative linkers between the Trigger and Effector (Drug) in a tripartite prodrug system **[Scheme 1]**.[1,2] These self-immolative linkers are particularly required when the activating enzyme is an endopeptidase, a peptide-cleaving

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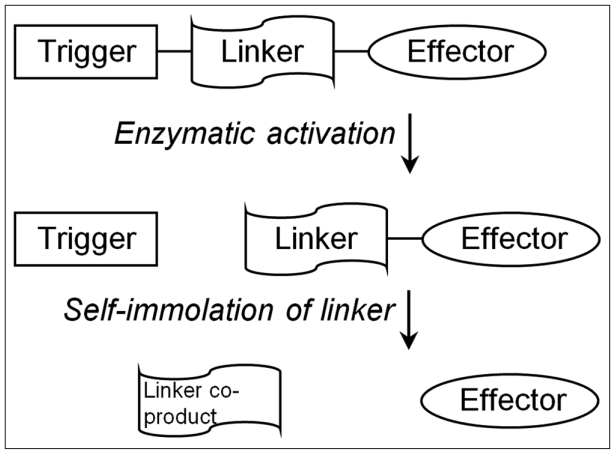


Scheme 1: Schematic representation of the release of drugs from a tripartite prodrug system. The trigger linker unit comprises the group which masks the pharmacophore of the drug (effector)

enzyme that can only hydrolyse amide bonds between amino-acids. In this case, it is essential that the self-immolative linker resembles an amino-acid or short peptide sufficiently to be recognized and bound by the activating enzyme.[7,8] In peptides with free *N*-terminal amines, the *N*-terminal amino acid pair can cyclise slowly to give a diketopiperazine (DKP), expelling the C-terminal remainder of the peptide as a leaving group. The rates of formation of the DKPs depend on the proportion of the dipeptide in the reacting *cis* conformation. Most peptide sequences adopt only the *trans*-conformation because it is energetically favorable. Peptide bonds to proline (Pro) are tertiary amides and the two conformers have approximately the same energy, leading to similar concentrations at equilibrium. *Pseudo*prolines (ΨPro) are proline analogues in which the γ-CH_2 group of the prolyl ring has been replaced by oxygen or sulfur atoms.[9] They have been reported to prevent peptide aggregation and self-association, thus improving the solvation and coupling kinetics in the assembly of peptides.[10,11] Most ΨPro have two methyl groups at the bridging methylene (2,2-dimethylthiazolidine-carboxylates [Dmt] and 2,2-dimethyl oxazolidine-carboxylates [Dmo]). Due to the presence of the *gem*-dimethyl unit,[12-16] these dipeptides are in (almost) exclusively the *cis*-amide conformation. This places the *N*-terminal primary amine appositely for nucleophilic attack at the ΨPro-carbonyl. This attack releases the amine-containing (model) drug and forms the corresponding DKPs. Previously, we have studied the rates of the expulsion of a model drug from a candidate series of molecular clips, the *N*-aminoacyl-2,2-dimethylthiazolidine-4-carboxamides (Aaa-Dmt). Structural studies[2] on the corresponding DKPs, 6-alkyl-3,3-dimethyltetrahydrothia zolo[3,4-*a*]pyrazine-5,8-diones, helped to rationalise the dependence of the relative rates of the cyclisation reaction on the bulk of the side chain of the *N*-terminal amino acid and on the relative stereochemical configuration of the dipeptide. Interestingly, the ring-closure is markedly faster with L-configuration at the *N*-terminal amino acid and *S*-configuration at the thiazolidine 4-C (corresponding to a D-amino-acid).[2] Aminoacyl-L-Dmt units are shown to be highly effective self-immolative molecular clips for incorporation into prodrug linkers designed for cleavage by endopeptidases but the thiazolidine ring has to be introduced early in the syntheses, causing many difficulties.[2] A solution to this problem could be the use of the oxygen analogues, the *N*-aminoacyl-2,2-dimethyloxazolidine-4-carboxamides (Aaa-Dmo) as this heterocycle could be introduced later in the synthesis. Before studying the rates of the expulsion of a model drug with these *pseudo*-dipeptides, the corresponding DKPs (6*S*,8a*R/S*-6-alkyl-3,3-dimethyltetrahydrooxazolo[3,4-*a*]pyrazine-5,8-diones) derived from L-Ala, L-Leu and L-Val have been synthesisedand characterized by nuclear magnetic resonance (NMR) and X-ray crystallography for structural studies.

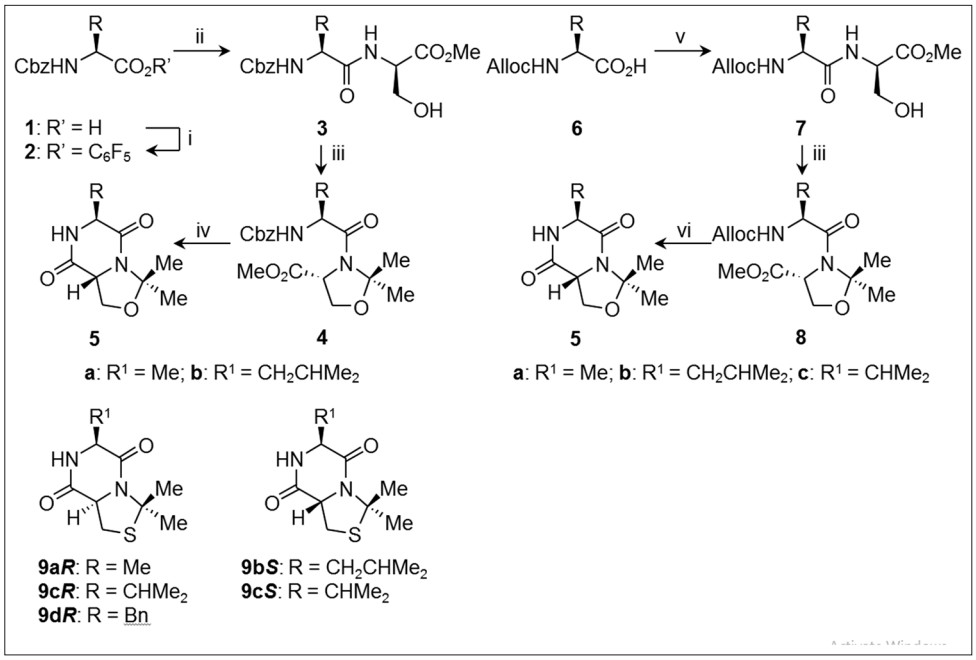
RESULTS AND DISCUSSION

Chemical synthesis

The target DKPs containing the Dmo unit were synthesized by cyclisation of appropriate dipeptide methyl esters **[Scheme 2].** Appropriate selection of the *N*-terminal protecting group is critical for this synthetic sequence, as the 2,2-dimethyl oxazolidine unit is constructed under acidic conditions but is notoriously sensitive to acid, and the *C*-terminal methyl ester would be cleaved by the aqueous base. *N*-Cbz and *N*-Alloc satisfy the criteria for suitable protecting groups in the synthesis of the dipeptides and hence the DKPs.

In the first route, Cbz-L-AlaOH **1a** and Cbz-L-LeuOH **1b** were converted to their pentafluorophenyl active esters **2a,b** in the usual way with dicyclohexylcarbodiimide. These were coupled with the methyl ester of D-serine to provide the protected dipeptides **3a,b**. The oxazolidine ring was then constructed by condensation with 2,2-dimethoxypropane,[16] catalysed by toluenesulfonic acid, providing the protected Dmo dipeptides **4a*R*** and **4b*R*** in moderate yields. Deprotection of Cbz by catalytic hydrogenation gave the free base which cyclised spontaneously to afford the required DKPs**5a*R*** and **5b*R*** in very good yield.

In the second route, Alloc-protected L-amino-acids (L-Ala, L-Val, L-Leu) **6a-c** were coupled using the 1-hydroxybenzotriazole (HOBt)/*N,N'*-dicyclohexylcarbodiimide (DCC) method with the methyl ester of D-serine to provide the protected dipeptides **7a-*cR*** and with the methyl ester of L-serine to provide the protected dipeptides **7a-*cS***. The oxazolidine ring was then constructed by condensation with 2,2-dimethoxypropane in boiling toluene, catalysed by toluenesulfonic acid, providing the protected Dmo dipeptides **8a-*cR***and **8a-*cS*** in moderate yields. Exploring methods of removal of the N-Alloc protection, treatment with tetrakis(triphenylphosphine) palladium(0) and 1,4-diazabicyclo[2.2.2]octane (DABCO) gave the desired DKPs**5a-c*R*** and **5a-c*S*** in 20 min in moderate yields. Replacement of the base by Me_2NSiMe_3 gave the DKPs in 10 min with markedly improved yields. In each case, the intermediate deprotected dipeptide methyl ester was never detected, as it cyclised to the DKP too rapidly.



Scheme 2: Synthesis of tetrahydrooxazolo[3,4-*a*]pyrazine-5,8-diones 5a,b,c *R* and *S*. *Reagents*: (i) *N,N'*-dicyclohexylcarbodiimide (DCC), C_6F_5OH, ethyl acetate (EtOAc), (ii) D-SerOMe.HCl, Et_3N, CH_2Cl_2, 75–77%, (iii), $Me_2C(OMe)_2$, 4-methylbenzenesulfonic acid hydrate, 38%, (iv) H_2, Pd/C, EtOAc, 37–100%; v, L-SerOMe.HCl *or* D-SerOMe. HCl, DCC, 1-hydroxybenzotriazole, Et_3N, CH_2Cl_2, (vi) $Pd(PPh_3)_4$, Me_2NSiMe_3, CH_2Cl_2, H_2O, 40–100% or $Pd(PPh_3)_4$, 1,4-diazabicyclo[2.2.2]octane, CH_2Cl_2, 54%. *R* and *S* in compound numbers refer to the configurations of the Ser unit of 3,7, of 4-C of the oxazolidinone of 8 or of 8*a*-C of 5. Structures of analogous 6*S*,8a*R/S*-6-alkyl-3,3-dimethyltetrahydrothiazolo[3,4-*a*] pyrazine-5,8-diones reported previously[2]

Conformational studies

L,S-Diastereomeric series - NMR studies

In the L,*S*-diastereomeric series, neither *cyclo*-L-Ala-*S*-Dmo **5a*S*** nor *cyclo*-L-Leu-*S*-Dmo **5b*S*** formed crystals of a quality suitable for crystallography and *cyclo*-L-Val-*S*-Dmo **5c*S*** was an oil. This contrasts with the corresponding DKPs derived from *R*-5,5-dimethylthiazoline-4-carboxylic acid (*R*-Dmt), where *cyclo*-L-Ala-*R*-Dmt **9a*R*** and *cyclo*-L-Val-*R*-Dmt **9c*R*** had formed excellent crystals for crystallographic studies;[2] in these Dmt derivatives, *R* configuration corresponds to *S* configuration in the Dmo compounds, due to the Cahn, Ingold, Prelog conventions. However, NOESY spectroscopy and examination of the ^{1}H-^{1}H NMR coupling constants allowed some inferences to be made about their conformation in solution.

The NOESY spectrum of **5a*S*** showed NOE correlation between the downfield methyl singlet (δ 1.56) and the 6-H (δ 4.08); thus this downfield singlet signal is due to the methyl on the lower (α) face of the molecule **[Figure 1]**. NOE correlation was also seen between the 1-H triplet (δ 4.02) and the α-face methyl singlet at δ 1.56; thus the signal at δ 4.02 is due to the 1-H on the α-face. Other NOE correlations were seen between the 6-H signal (δ 4.08) and the multiplet at δ 4.34, which comprises the signals for 8a-H and 1_β-H. As this 1-H is on the β–face, the correlation must be between 8a-H and 6-H, and they are shown to be 1,4-diaxial and *cis*, an arrangement only available in a boat conformation. Similar observations were also made for **5b*S*** and **5c*S***. Interestingly, Karle also reported a boat conformation for the DKP ring in *cyclo*-L-Leu-L-Pro in the crystalline state.[17]

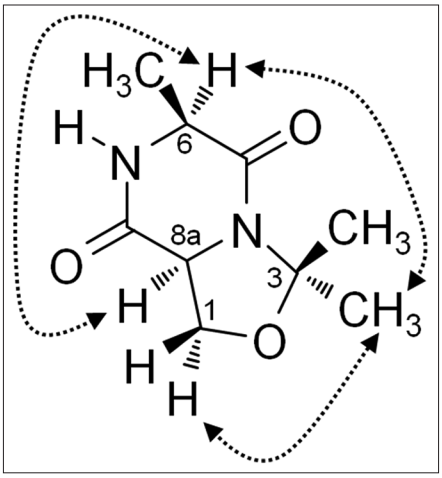


Figure 1: Selected NOE correlations for protons on the α-face of *cyclo*-L-Ala-*S*-2,2-dimethyloxazolidine-carboxylates 5a*S*

The boat conformation of the DKP ring was confirmed by the presence of a five-bond coupling $^5J = 1.2$ Hz for **5a*S*** and **5b*S*** and $^5J = 1.5$ Hz for **5c*S*** between the axial 8a-H and the axial 6-H. Therefore, the amino acid side chains (CH_3 for **5a*S***, $CHCH_2Me_2$ for **5b*S*,** and $CHMe_2$ for **5c*S***) are in *pseudo*-equatorial positions and are remote from the geminal dimethyl unit at 6-C. As is the case for the Dmt analog, the oxazolidine ring is in a half-chair conformation, with a *trans*-diaxial coupling between the axial 8a-H and the 1_β-H proton with $^3J = 12.1$ Hz for **5a*S*** (the corresponding coupling in *cyclo*-L-Ala-*R*-Dmt is ^3J = 10.2 Hz). Unfortunately,

the signals for 1_{α}-H and 8a-H overlapped, precluding measurement of the coupling constant ^{3}J between them. Similar couplings were observed for **5b*S*** and for **5c*S*** with ^{3}J = 11.7 Hz (the corresponding coupling constant in *cyclo*-L-Val-*R*-Dmt **9c*R*** is also ^{3}J = 11.7 Hz). 8a-H is on the α-face; thus, the only half-chair consistent with the large vicinal *trans*-diaxial coupling constants between 1-H_{β} and 8a-H is the one with (3-C)-(4-N)-(8a-C)-(1-C) in one plane and 2-O being the atom out-of-plane **[Figure 2a]**. These conformations are very similar to those found from the crystal structure of the *R*-Dmt analogues.[2]

Some deductions could also be made about the conformations of the side chains at the 6-position in **5b*S*** and **5c*S***. First, the chemical shift of the 6-H depended on the size of the side chains, with increasing local steric bulk (Me<CH_2CHMe_2<$CHMe_2$) moving this resonance upfield (δ 4.08 for **5a*S***, δ 3.98 for **5b*S*,** and δ 3.90 for **5a*S***). Second, in *cyclo*-L-Val-*S*-Dmo **5c*S***, an NOE correlation was observed between the signal for the *pseudo*-axial 3_{β}-Me (δ 1.63) and one of the methyl groups of the 6-isopropyl unit (δ 0.91); the couplings to 6-H were also unusually small (in comparison with those in **5a*S*** and **5b*S***), with ^{3}J = 1.5 Hz for the transannular coupling to 8a-H (*vide supra*) and ^{3}J = 2.3 Hz for the coupling with 2'-H. Taken together, these data point to the conformation shown in **Figure 2b** for **5c*S***, with the pro-*R* side chain methyl (δ 0.91) located over the DKP ring close to the shielding cone of the 5-carbonyl.

L,RDiastereomeric series - crystallographic studies

Crystallographic quality crystals of *cyclo*-L-Ala-*R*-Dmo **5a*R***, *cyclo*-L-Leu-*R*-Dmo **5b*R*,** and *cyclo*-L-Val-*R*-Dmo **5c*R*** were grown from ethyl acetate (EtOAc)/petroleum ether and the X-ray crystal structures were determined. The Dmt analogues **9b*S*** and **9c*S* [Scheme 2]** had failed to provide crystals of suitable quality.[2] **Figure 3** shows the intermolecular hydrogen-bonding (H-bonding) pattern evident in the crystals. For *cyclo*-L-Ala-*R*-Dmo **5a*R*** and *cyclo*-L-Val-*R*-Dmo **5c*R***, the intermolecular H-bonding forms a ribbon through the crystal, with the NH being H-bonded to the secondary amide carbonyl of the adjacent molecule. The tertiary amide carbonyl (from the dimethyloxaproline unit) does not participate in H-bonding. The intermolecular H-bonding motif in *cyclo*-L-Leu-*R*-Dmo **5b*R*** is different from that in the other crystals, in that the H-bonding pairs are formed with the N-H and the tertiary amide carbonyl of the adjacent molecule. In that case, the secondary amide carbonyl does not participate in H-bonding.

Within an individual molecule of *cyclo*-L-Ala-*R*-Dmo **5a*R***, the DKP ring is in a flattened conformation, with the oxazolidine in a half-chair. This ring conformation places the geminal methyl groups in equatorial and axial positions, and the 6-methyl occupies a sterically open region of space **[Figure 2c]**; notably, this 6-methyl group is remote in space from the geminal dimethyl unit. The half-chair is different from that seen in the diastereoisomer **5a*S***, in that

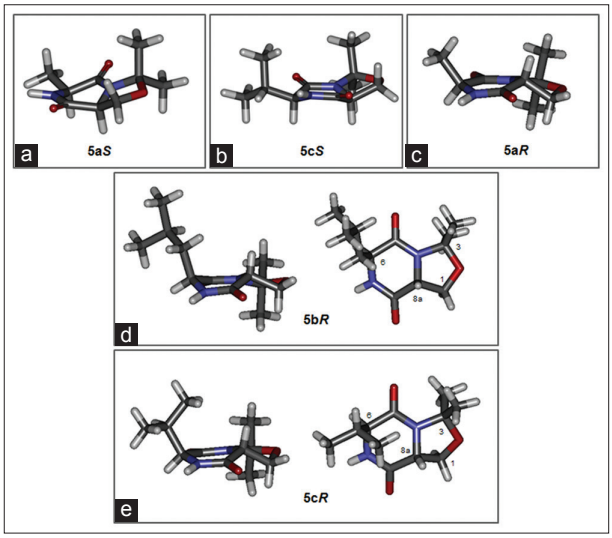


Figure 2: (a) MM2-minimized structure of *cyclo*-L-Ala-*S*-2,2-dimethyloxazolidine-carboxylates (Dmo) 5a*S*. (b) Solution conformation of *cyclo*-L-Val-*S*-Dmo 5c*S* deduced from nuclear magnetic resonance studies. (c) X-ray crystal structure, showing solid-state conformation of *cyclo*-L-Ala-*R*-Dmo 5a*R*. (d) X-ray crystal structure, showing solid-state conformation of *cyclo*-L-Leu-*R*-Dmo 5b*R*. (e) X-ray crystal structure, showing solid-state conformation of *cyclo*-L-Val-*R*-Dmo 5c*R*. In d and e, the upper "side" views show the conformations around the diketopiperazines ring, whereas the lower "top" views show the conformations about the 6-C side chain bond for 5b*R* and 5c*R*

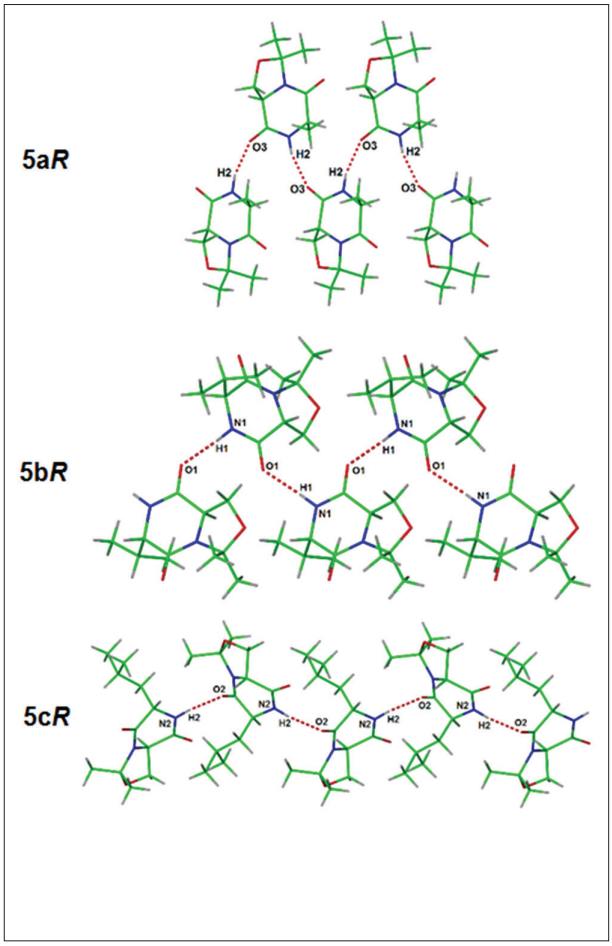


Figure 3: Intermolecular hydrogen-bonding patterns in crystals of *cyclo*-L-Ala-*R*-2,2-dimethyloxazolidine-carboxylates (Dmo) 5a*R*,*cyclo*-L-Leu-*R*-Dmo 5b*R*, and *cyclo*-L-Val-*R*-Dmo 5c*R*

(2-O)-(3-C)-(4-N)-(8a-C) of **5a*R*** are in one plane with 1-C being the atom out-of-plane folded away from 8a-H.

In *cyclo*-L-Leu-*R*-Dmo **5b*R***, as the bulk of the side chain at 6-C is increased, the DKP ring becomes a flattened boat, placing the 6-isobutyl substituent in a *pseudo*-axial position. The same half-chair for the oxazolidine is observed for **5b*R*** as for the Ala analogue **5a*R***. The same flattened boat and half-chair were shown by NMR for the Dmt analogue **9b*S***. The (6-C)—(2'-C) bond is in the expected staggered conformation, with the bulky isopropyl group pointing away from the DKP ring **[Figure 2d]**.

In the structure of *cyclo*-L-Val-*R*-Dmt**5c*R***, the isopropyl group at 6-C is more sterically bulky than the isobutyl unit of **5b*R*** in the critical region close to the DKP ring. However, the DKP ring moves toward planarity, and its conformation is very similar to that of the Ala analogue **5a*R***. The same half-chair, with the 1-C out-of-plane, is preserved **[Figure 2e]**. The overall conformation resembles that suggested for its Dmt analogue **9c*S*** by NMR studies.[2] It is likely that steric repulsion between the 3_{β}-Me and the isopropyl group of **5c*R*** drives this flattening of the DKP ring, particularly as the conformation of the (6-C)—(2'-C) bond is *gauche* staggered, with one of the methyl groups of the isopropyl lying over the DKP ring, i.e. inclined toward the 3_{β}-Me. This conformation of the side chain contrasts with that deduced by Young *et al*.[18] for the analogous *cyclo*-L-Pro-D-Val from NMR data.

*L,*R-*Diastereomeric series - NMR studies*

The conformations of the L,*R* series solution in $CDCl_3$ were studied by NOESY spectroscopy and examination of ^{1}H-^{1}H NMR coupling constants. Notably, whereas the 3-Me signals had been well separated in the *cyclo*-L-Aaa-*S*-Dmo series (Δ_{δ} 0.06 ppm. for **5a*S***, **5b*S,*** and **5c*S***), the corresponding signals for the members of the *cyclo*-L-Aaa-*R*-Dmo series were almost coincident, which precluded many of the planned NOE measurements. Other examples of overlapping signals were also encountered.

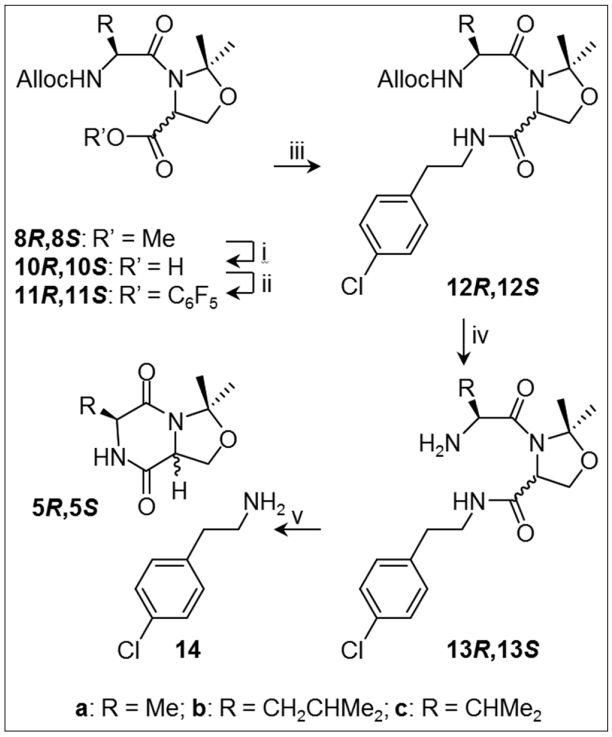
In the ^{1}H NMR spectrum of **5a*R***, the signals for 1_{α}-H, 1_{β}-H, and 8a-H all formed triplets with J = 8.2 Hz; thus, the geminal ^2J between 1_{α}-H and 1_{β}-H is unusually small. The HSQC spectrum allowed assignment of the triplet at δ 3.95 as arising from 1_{α}-H or 1_{β}-H but the configurational identity could not be confirmed. The other 1-H and 8a-H formed triplets at δ 4.33 and δ 4.36, but the individual assignments could not be made. In the corresponding spectra of **5b*R*** and **5c*R***, the same sets of three triplets were observed for the two 1-H protons and 8a-H; These consistent observations suggest that the conformations of the half-chair oxazolidine rings of these three analogues are almost identical, as seen in the solid-state structures.

The coupling constants between 6-H and the adjacent 7-H (N-H) in this diastereomeric series are large, with ^3J = 4.3 Hz for **5a*R***, ^3J = 6.6 Hz for **5b*R,*** and ^3J = 4.4 Hz for **5c*R***. In **5a*R*** and **5c*R***, these values are inconsistent with boat conformations of the DKP rings but indicate that the DKPs in this series are likely to be flattened markedly from the boat form. The much larger coupling constant in **5b*R*** shows that the DKP ring is more boat-like in this molecule. As expected, 8-aH did not couple with 6-H in any compound in this series, since these protons are now *trans* and no longer 1,4-diaxial. These observations suggest that the solution conformations of the rings resemble closely those observed in the solid-state. Interestingly, Cavelier *et al*.[19] found only one type of pucker of the five-membered rings in analogous bicyclic DKPs. In 2,2-dimethyltetrahydrosilazolo[3,4-*a*] pyrazine-5,8-dione (*cyclo*-Gly-(γ-(dimethylsila)Pro) and tetrahydrothiazolo[3,4-*a*]pyrazine-5,8-dione (*cyclo*-Gly-(γ-thia)Pro), the 1-C is the ring-atom which is out-of-plane. In the present work, we found this 1-C to be the atom out-of-plane in the **5a*R***, **5b*R***, and **5c*R*** series but **5a*S***, **5b*S***, and **5c*S*** adopted a different ring-pucker with the 2-O out-of-plane.

Some inferences could also be drawn about the conformations of the 6-side-chains in solution. In **5b*R***, the coupling between 6-H and the 2'-*pro*-*S*-H is ^3J = 9.0 Hz, whereas the coupling between 6-H and the 2'-*pro*-*R*-H is approximately zero. Applying the Karplus relationship shows that these coupling constants are only consistent with a conformation around the (6-C)—(2'-C) bond similar to that in the crystal but very slightly twisted to place the 2'-*pro*-*R*-H at *ca.* 90° dihedral angle to 6-H. In **5c*R***, the observation of a ^3J = 5.8 Hz coupling between 6-H and 2'-H shows that these protons cannot be antiperiplanar about the (6-C)—(2'-C) bond. This contrasts with the deduction by Young *et al*.[18] for *cyclo*-L-Pro-D-Val from NMR data suggesting that these protons are antiperiplanar in this latter DKP. The 2'-methyl group signals resonate as ^3J = 6.6 Hz doublets at δ 0.97 and δ 1.01 and were distinguished in the NOESY spectrum. The downfield signals showed an NOE to the 6-H signal at δ 3.65 and the upfield signal at δ 0.97 showed an NOE to the multiplet at δ 4.3 containing the 8a-H and one 1-H. Since both 1-H protons are too remote from either 2'-Me for an NOE interaction to be evident, this NOE must be between 8a-H and the methyl signal. These NOE observations are only consistent with a conformation of the side chain in solution which is very similar to that in the crystal, with the *pro*-*S* methyl over the DKP ring and close to 8a-H. This conformation places this *pro*-*S* methyl remote from 6-H. However, it also places the *pro*-*R* 2'-Me close to 6-H but remote from 8a-H. The signals are, therefore, assigned as δ 0.97 (*pro*-*S* 2'-Me) and δ 1.01 (*pro*-*R* 2'-Me).

Cyclisation studies

The rates of ring-closure of the Dmt-containing dipeptides are independent of *pseudo*-proline *cis*-*trans* interconversion but depend on the relative configurations of the two stereocentres, with the L,*S*-Dmt dipeptide amides cyclising much more rapidly than their L,*R* diastereoisomers.[1] In view of the similarity of the conformation in the present Dmo series, it is likely that similar effects prevail during the cyclisation of L,*R*/*S*-Dmo dipeptide amides with the L,*R*-Dmo dipeptide amides cyclising much more rapidly than their L,*S*-diastereoisomers. To confirm this hypothesis, Alloc-Val-*R*/*S*-Dmo dipeptide amides **12*R*** and **12*S*** were synthesised and the free-base dipeptide amides **13*R*** and **13*S*** were revealed and tested for the release of a model drug **[Scheme 3].**



Scheme 3: Synthesis of diastereomeric Alloc dipeptide amides 12*R* and 12*S* carrying the model drug, deprotection of 12*R* and 12*S* and spontaneous cyclisation of dipeptides 13*R* and 13*S*, forming diketopiperazines 5*R* and 5*S* and amine 14. *Reagents*: (i) NaOH, H_2O, MeOH, (ii) *N,N'*-dicyclohexylcarbodiimide, C_6F_5OH, ethyl acetate, (iii) 4-ClPh$(CH_2)_2NH_2$.HCl, Et_3N,CH_2Cl_2, (iv) $Pd(PPh_3)_4$, Me_2NSiMe_3, CH_2Cl_2, water; $CDCl_3$, (v) spontaneous

2-(4-Chlorophenyl)ethylamine **14** was selected as a model for amine-containing drugs, where the amines are key features of the pharmacophores. Mild hydrolysis of the intermediate dipeptide esters **8*R*** and **8*S*** exposed the carboxylic acids **10**, which were converted to their pentafluorophenyl active esters **11** for coupling with **14**. The Alloc protecting groups were removed rapidly under neutral conditions ($(Ph_3P)_4Pd$ with either Me_3SiNMe_2 or DABCO) to provide the dipeptide amides **12**, which are models of the [dipeptide molecular clip]–[drug] for use in measuring rates of cyclisation to **5** and expulsion of **14**. Experiments using HPLC to monitor the rates of these cyclisations/drug expulsions were uninformative, due to difficulties caused by the presence of the Pd, and only served to confirm cyclisation was rapid. However, the reactions were studied very effectively by generating the dipeptide amides **13*R*** and **13*S*** in NMR tubes and following the rates of cyclisation by ^{1}H NMR spectroscopy; examples of the graphs of the cyclisation/release reactions in $CDCl_3$ are shown in **Figure 4**. Although the conditions are non-physiological and non-aqueous, conclusions can be drawn about the relative rates of cyclisation/release of **14**. The dipeptide amide **13c*R*** containing *S*-Dmo cyclised faster ($t_{½}$ *ca.* 40 h) than did **13c*S*** containing *R*-Dmo ($t_{½}$>12 d). Other diastereomeric pairs of dipeptide amides showed similar differences in rates of cyclisation/expulsion of **14** (data not shown). The Leu dipeptide amide **13b*R*** also appeared to cyclise slightly slower than the corresponding Val dipeptide amide **13c*R***.

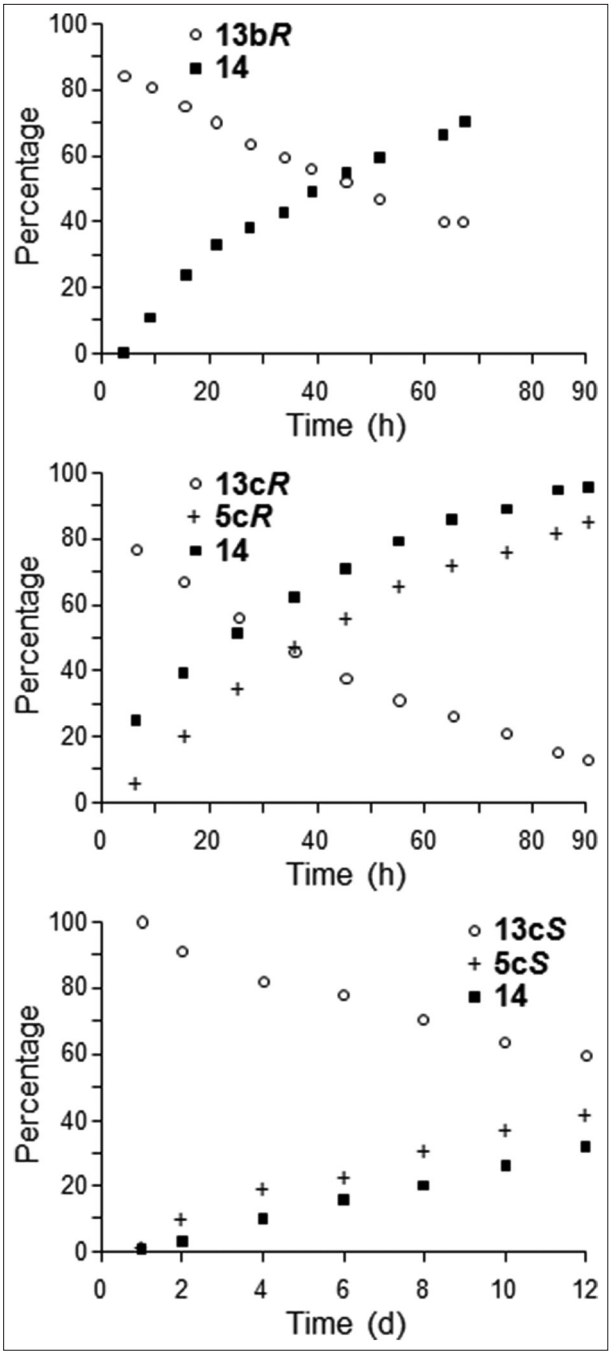


Figure 4: Graphs of preliminary study on cyclisation of diastereomeric dipeptide amides 13c*R* and 13c*S* and homologue 13b*R*. Samples were dissolved in $CDCl_3$ (ca. 40 mM), and the solutions were kept at 20°C; ^{1}H nuclear magnetic resonance spectra were recorded at the intervals shown, and the amounts of the dipeptides remaining and the product diketopiperazines and 14 were estimated by integration of appropriate well-resolved distinct peaks

These observations are consistent with the effects seen in the Dmt series (the cyclisation of which in water was studied by HPLC).[2] Although the Dmo dipeptide amides appear, at first sight, to react slower than their Dmt analogues, this is probably an artifact of the different polarities of the solvents used. Further experiments will be needed to translate these preliminary findings into the aqueous environment.

Toxicity study

When designing prodrugs, it is important to ensure that any fragments released from the self-immolation of the linker are essentially non-toxic. The cytotoxicity of *cyclo*-L-Leu-*R*-Dmo **5b*R*** was determined using the HT29 human colon carcinoma tumour cell line as a surrogate for toxicity toward normal human cells. Cell survival was assessed by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) (MTS) method, after incubation of the compound with the cells for 3 d. Compound **5b*R*** was non-toxic to the cells up to the maximum concentration tested, 0.5 mM, pointing to the likely safety of this DKP derived from an unnatural amino acid.

EXPERIMENTAL SECTION

General

^{1}H NMR spectra were recorded on Varian GX270 or EX400 spectrometers. Infrared spectra were recorded on a Perkin-Elmer 782 spectrometer as KBr discs, unless otherwise stated. Mass spectra were obtained using high-resolution electrospray ionisation in the positive ion mode, unless otherwise stated. The chromatographic stationary phase was silica gel. Solutions in organic solvents were dried with magnesium sulfate. Solvents were evaporated under reduced pressure. The aqueous sodium hydrogen carbonate and brine were saturated. Experiments were conducted at ambient temperature, unless otherwise stated. Melting points were measured with a Thermo Galen Kofler block.

*N-(N-Phenylmethoxycarbonyl-L-alanyl)-D-serine methyl ester (3a*R*)*

Cbz-L-AlaOPFP **2a** (823 mg, 2.1 mmol) was stirred with D-SerOMe.HCl (310 mg, 2.0 mmol) and Et_3N (606 mg, 6.0 mmol) in CH_2Cl_2 (20 mL) for 16 h. The solution was washed with water, aq. Na_2CO_3 (1.0 M) and aq. HCl (1.0 M). Evaporation afforded **3a*R*** (497 mg, 77%) as a colorless gum: ^{1}H NMR ($CDCl_3$): δ 1.40 (3 H, d, *J* = 7.0 Hz, Ala Me), 3.76 (3 H, s, OMe), 3.94 (2 H, m, Ser β-H_2), 4.26 (1 H, qn, *J* = 6.7 Hz, Ala α-H), 4.60 (1 H, m, Ser α-H), 5.08 (1 H, d, *J* = 14 Hz, PhCH), 5.09 (1 H, d, *J* = 14 Hz, PhCH), 5.34 (1 H, d, *J* = 6.3 Hz, Ala NH), 7.03 (1 H, d, *J* = 7.0 Hz, Ser NH).

(N-(N-Phenylmethoxycarbonyl)-D-serine methyl ester(3bR)

Cbz-L-LeuOPFP **2b** (980 mg, 2.2 mmol) was stirred with D-SerOMe.HCl (330 mg, 2.1 mmol) and Et_3N (612 mg, 6.0 mmol) in CH_2Cl_2 (20 mL) for 16 h. The mixture was washed with water, aq. Na_2CO_3 (1.0 M) and aq. HCl (1.0 M). Evaporation afforded **3b*R*** (580 mg, 75%) as a white solid: mp 100–103°C. ^{1}H NMR ($CDCl_3$): δ 0.93 (6 H, d, *J* = 6.3 Hz, Leu Me_2) 1.55 (1 H, m, Leu γ-H), 1.68 (2 H, m, Leu β-H_2), 3.7 (1 H, t, *J* = 4.7 Hz, OH), 3.80 (3 H, s, OMe), 3.90 (2 H, m, Ser β-H_2), 4.21 (1 H, m, Leu α-H), 4.62 (1 H, m, Ser α-H), 5.1 (2 H, s, $PhCH_2$), 5.20 (1 H, d, *J* = 6.3 Hz, Leu NH), 7.01 (1 H, d, *J* = 7.8 Hz, Ser NH), 7.33 (5 H, m, Ph-H_5).

*Methyl N-(N-phenylmethoxycarbonyl-L-alanyl)-*R*-2,2-dimethyl-1,3-oxazolidine-4-carboxylate(4a*R*)*

Cbz-L-Ala-D-SerOMe **3a*R*** (492 mg, 1.35 mmol) was heated with 2,2-dimethoxypropane (4.0 mL) and 4-methylbenzenesulfonic acid hydrate (TsOH) (28 mg) in CH_2Cl_2 (13.5 mL) under reflux for 16 h. The solution was washed with aq. $NaHCO_3$ (5%) and water. Evaporation and chromatography (EtOAc) gave **4a*R*** (191 mg, 38%) as a colourless gum: ^{1}H NMR ($CDCl_3$): δ 1.30 (3 H, d, *J* = 6.7 Hz, Ala Me), 1.54 (3 H, s, oxazolidine 2-Me), 1.65 (3 H, s, oxazolidine 2-Me), 3.80 (3 H, s, OMe), 4.09 (2 H, m, Ala α-H and oxazolidine 5-H), 4.16 (1 H, d, *J* = 9.4 Hz, oxazolidine 5-H), 5.05 (1 H, d, *J* = 12.5 Hz, PhCH), 5.10 (1 H, d, *J* = 12.5 Hz, PhCH), 5.12 (1 H, dd, *J* = 9.4, 2 Hz, oxazolidine 4-H), 5.27 (1 H, d, *J* = 8.2 Hz, Ala NH), 7.35 (5 H, m, Ph-H_5).

*Methyl N-(N-phenylmethoxycarbonyl-L-leucyl)-*R*-2,2-dimethyl-1,3-oxazolidine-4-carboxylate(4b*R*)*

Cbz-L-Leu-D-SerOMe **3b*R*** (570 mg, 1.6 mmol) was heated with 2,2-dimethoxypropane (10 mL) and TsOH (31 mg) in CH_2Cl_2 (10 mL) under reflux for 16 h. The solution was washed with aq. $NaHCO_3$ (5%) and water. Evaporation and chromatography (EtOAc) gave **4b*R*** (191 mg, 38%) as pale yellow crystals: mp 77–79°C; ^{1}H NMR ($CDCl_3$): δ 0.82 (3 H, d, *J* = 6.3 Hz, Leu Me), 0.89 (3 H, d, *J* = 6.7 Hz, Leu Me), 0.9-1.4 (3 H, m, Leu β,β,γ-H_3), 1.53 (3 H, s, oxazolidine 2-Me), 1.66 (3 H, s, oxazolidine 2-Me), 3.80 (3 H, OMe), 4.10 (1 H, m, Leu α-H), 4.15 (1 H, dd, *J* = 6.3, 3.1 Hz, oxazolidine 4-H), 4.17 (1 H, m, oxazolidine 4-H), 5.07-5.12 (3 H, m, $PhCH_2$ and oxazolidine 4-H), 5.15 (1 H, d, *J* = 9.0 Hz, Leu NH), 7.32 (5 H, m, Ph-H_5).

*6S,8a*R*-3,3,6-Trimethyltetrahydrooxazolo[3,4-a]pyrazine-5,8-dione(5a*R*) Method A*

Compound **4a*R*** (191 mg, 520 μmol) was stirred vigorously under hydrogen with palladium on charcoal (10%, 100 mg) in MeOH (15 mL) for 16 h. Filtration (Celite®), chromatography (EtOAc/hexane 1:1) and recrystallisation (EtOAc/hexane) gave **5a*R*** (80 mg, 89%) as white needles: mp 151–153°C; ^{1}H NMR ($CDCl_3$) δ 1.43 (3 H, d, *J* = 7.1 Hz, 6-Me), 1.61 (3 H, s, 3-Me), 1.62 (3 H, s, 3-Me), 3.95 (1 H, qd, *J* = 7.0, 4.3 Hz, 6-H), 3.95 (1 H, t, *J* = 8.2 Hz, 1-H), 4.33 (1 H, t, *J* = 8.2 Hz, 1-H or 8a-H), 4.36 (1 H, t, *J* = 8.2 Hz, 8a-H or 1-H), 6.60 (1 H, s, 7-H); ^{13}C NMR ($CDCl_3$) (HMBC/HSQC) δ 19.63 (6-Me), 23.41 (3-Me), 25.45 (3-Me), 53.94 (6-C), 55.70 (8a-C), 96.13 (3-C), 164.65 (5-C), 167.50 (8-C); HRESIMS *m/z* 221.0898 [M + Na]$^+$ ($C_9H_{14}N_2NaO_3$ requires 221.0902).

*6S,8aR-3,3,6-Trimethyltetrahydrooxazolo[3,4-a]pyrazine-5,8-dione(5a*R*) Method B*

Alloc-L-Ala-*R*-DmoOMe **8a*R*** (314 mg, 1.0 mmol) was stirred with $Pd(PPh_3)_4$ (60 mg, 50 mmol) and Me_3SiNMe_2 (354 mg, 3.0 mmol) in CH_2Cl_2(5.0 mL) for 10 min. Water (1.0 mL) was added. After 10 min, the reaction was quenched with aq. $NaHCO_3$ (5%) and the mixture was extracted twice with CH_2Cl_2. Drying, evaporation and chromatography (hexane/EtOAc 1:1) gave **5a*R*** (80 mg, 40%), with properties as above.

6S,8aR-3,3,6-Trimethyltetrahydrooxazolo[3,4-a]pyrazine-5,8-dione(5aR) Method C

Alloc-L-Ala-R-DmoOMe **8a*R*** (314 mg, 1.0 mmol) was stirred with $Pd(PPh_3)_4$ (120 mg, 100 mmol) and DABCO (560 mg, 5.0 mmol) in CH_2Cl_2 (5.0 mL) for 20 min. The reaction was quenched with aq. $NaHCO_3$ (5%) and the mixture was extracted twice with CH_2Cl_2. Drying, evaporation and chromatography (hexane/EtOAc 1:1) gave **5a*R*** (108 mg, 54%), with properties as above.

6S,8aS-3,3,6-Trimethyltetrahydrooxazolo[3,4-a]pyrazine-5,8-dione (5aS) Method A

Alloc-L-Ala-*S*-DmoOMe **8a*S*** was treated with $Pd(PPh_3)_4$ and Me_3SiNMe_2, as for the synthesis of **5a*R*** (Method B), to give **5a*S*** (37%) as white crystals: mp 120–122°C. ^{1}H-NMR ($CDCl_3$) δ 1.43 (3 H, d, *J* = 7.0 Hz, 6-Me), 1.56 (3 H, s, 3_α-Me), 1.62 (3 H, s, 3_β-Me), 4.02 (1 H, t, *J* = 12.1 Hz, 1_β-H), 4.08 (1 H, qd, *J* = 7.0, 1.2 Hz, 6-H), 4.32 (1 H, dd, *J* = 12.1, 7.0 Hz, 1_α-H), 4.34 (1 H, ddd, *J* = 12.1, 7.0, 1.1 Hz, 8a-H), 6.80 (1 H, br, 7-H); ^{13}C-NMR (HMBC/HMQC) δ 15.28 (6-Me), 23.50 (3_α-Me), 25.30 (3_β-Me), 51.43 (6-C), 57.34 (8a-C), 65.11 (1-C), 95.88 (3-C), 164.35 (5-C), 168.64 (8-C); HRESIMS *m/z* 197.0937 $[M - H]^-$ ($C_9H_{13}N_2O_3$ requires 197.0932).

6S,8aS-3,3,6-Trimethyltetrahydrooxazolo[3,4-a]pyrazine-5,8-dione (5aS) Method B

Alloc-L-Ala-*S*-DmoOMe **8a*S*** was treated with $Pd(PPh_3)_4$ and DABCO, as for the synthesis of **5a*R*** (Method C), to give **5a*S*** (64%), with properties as above.

6S,8aR-3,3-Dimethyl-6-(2-methylpropyl) tetrahydrooxazolo[3,4-a]pyrazine-5,8-dione (5bR) Method A

Compound **4b*R*** was treated with hydrogen, as for the synthesis of **5a*R*** (method A), to give **5b*R*** (97%) as white crystals: mp 130–132°C. ^{1}H NMR ($CDCl_3$) δ 0.91 (3 H, d, *J* = 6.6 Hz, HC-C*H*$_3$), 0.98 (3 H, d, *J* = 6.6 Hz, HC-C*H*$_3$), 1.60 (3 H, s, 3-Me), 1.61 (3 H, s, 3-Me), 1.63 (2 H, m, C*H*$_2$CHMe$_2$), 1.72 (1 H, br nonet, *J* = 6.6 Hz, C*H*Me$_2$), 3.84 (1 H, dd, *J* = 9.0, 6.6 Hz, 6-H), 3.95 (1 H, br t, *J* = 8.0 Hz, 1-H), 4.28 (1 H, br t, *J* = 8.0 Hz, 1-H or 8a-H), 4.31 (1 H, br t, *J* = 8.0 Hz, 8a-H or 1-H), 7.25 (1 H, s, 7-H); ^{13}C NMR ($CDCl_3$) (HMBC/HSQC) δ 21.31 (HC-*C*H$_3$), 22.93 (HC-*C*H$_3$), 23.93 (3-Me), 24.45 (*C*HMe$_2$), 25.40 (3-Me), 42.03 (*C*H$_2$CHMe$_2$), 55.80 (8a-C), 56.55 (6-C), 65.63 (1-C), 96.05 (3-C), 164.57 (5-C), 167.87 (8-C); HRESIMS: 263.1371 $[M + Na]^+$ ($C_{12}H_{20}N_2NaO_3$requires 263.1371), 241.1552 $[M + H]^+$ ($C_{12}H_{21}N_2O_3$requires 241.1552).

6S,8aR-3,3-Dimethyl-6-(2-methylpropyl) tetrahydrooxazolo[3,4-a]pyrazine-5,8-dione (5bR) Method B

Alloc-L-Leu-R-DmoOMe **8b*R*** was treated with $Pd(PPh_3)_4$ and Me_3SiNMe_2, as for the synthesis of **5a*R*** (method B), to give **5b*R*** (quant.), with properties as above.

6S,8aS-3,3-Dimethyl-6-(2-methylpropyl) tetrahydrooxazolo[3,4-a]pyrazine-5,8-dione (5bS)

Alloc-L-Leu-*S*-DmoOMe **8b*S*** was treated with $Pd(PPh_3)_4$ and Me_3SiNMe_2, as for the synthesis of **5a*R*** (Method B), to give **5b*S*** (43%) as white crystals: mp 128–130°C. ^{1}H NMR ($CDCl_3$) δ 0.93 (3 H, d, *J* = 6.6 Hz, HC-C*H*$_3$), 0.98 (3 H, d, *J* = 6.6 Hz, HC-C*H*$_3$), 1.52 (1 H, ddd, *J* = 14.4, 11.9, 5.1 Hz, C*H*CHMe$_2$), 1.56 (3 H, s, 3_α-Me), 1.62 (3 H, s, 3_β-Me), 1.74 (1 H, m, C*H*Me$_2$), 2.00 (1 H, ddd, *J* = 14.4, 11.9, 5.1 Hz, C*H*CHMe$_2$), 3.98 (1 H, ddd, *J* = 9.4, 3.9, 1.2 Hz, 6-H), 4.03 (1 H, t, *J* = 11.7 Hz, 1_β-H), 4.31–4.70 (2 H, m, 1_α,8a-H$_2$), 4.70 (1 H, br, NH), 6.31 (1 H, br, 7-H); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 21.25 (HC-*C*H$_3$), 23.19 (HC-*C*H$_3$), 23.50 (3_β-Me), 24.68 (*C*HMe$_2$), 25.30 (3_α-Me), 38.04 (*C*H$_2$CHMe$_2$), 53.67 (6-C), 57.06 (8a-C), 65.12 (1-C), 95.95 (3-C), 164.17 (5-C), 168.40 (8-C); HRESIMS: 263.1372 $[M + Na]^+$ ($C_{12}H_{20}N_2NaO_3$ requires 263.1371), 241.1550 $[M + H]^+$ ($C_{12}H_{21}N_2O_3$ requires 241.1552).

6S,8aR-3,3-Dimethyl-6-(1-methylethyl) tetrahydrooxazolo[3,4-a]pyrazine-5,8-dione (5cR) Method A

Alloc-L-Val-*R*-DmoOMe **8b*R*** was treated with $Pd(PPh_3)_4$ and Me_3SiNMe_2, as for the synthesis of **5a*R*** (Method B), to give **5b*R*** (44%) as white crystals: mp 160–161°C. ^{1}H NMR ($CDCl_3$) δ 0.97 (3 H, d, *J* = 6.6 Hz, Val *pro-S* Me), 1.01 (3 H, d, *J* = 6.6 Hz, Val *pro-R* Me), 1.60 (6 H, s, 2 × 3-Me), 2.23 (1 H, d septet, *J* = 5.8, 6.6 Hz, C*H*Me$_2$), 3.65 (1 H, dd, *J* = 5.8, 4.4 Hz, 6-H), 3.88 (1 H, t, *J* = 11.6 Hz, 1-H), 4.29-4.35 (2 H, m, 1,8a-H$_2$), 7.17 (1 H, br, 7-H); ^{13}C-NMR ($CDCl_3$) (HMBC/HSQC) δ 17.73 (Val *pro-S* Me), 18.90 (Val *pro-R* Me), 23.30 (3-Me), 25.55 (3-Me), 32.95 (*C*HMe$_2$), 56.10 (8a-C), 63.73 (6-C), 65.90 (1-C), 96.21 (3-C), 163.58 (5-C), 167.75 (8-C); HRESIMS *m/z* 249.1209 $[M + Na]^+$ ($C_{11}H_{18}N_2NaO_3$ requires 249.1215).

6S,8aR-3,3-Dimethyl-6-(1-methylethyl) tetrahydrooxazolo[3,4-a]pyrazine-5,8-dione (5cR) Method B

Alloc-L-Leu-*R*-DmoOMe **8b*R*** was treated with $Pd(PPh_3)_4$ and DABCO, as for the synthesis of **5a*R*** (Method C), to give **5b*R*** (quant.), with properties as above.

6S,8aS-3,3-Dimethyl-6-(1-methylethyl) tetrahydrooxazolo[3,4-a]pyrazine-5,8-dione (5cS) Method A

Alloc-L-Val-*S*-DmoOMe **8c*S*** was treated with $Pd(PPh_3)_4$ and Me_3SiNMe_2, as for the synthesis of **5a*R*** (Method B), to give **5c*S*** (quant.) as a colourless oil: ^{1}H NMR ($CDCl_3$): δ 0.91 (3 H, d, *J* = 6.6 Hz, Val *pro-R* Me), 1.06 (3 H, d, *J* = 6.6 Hz, Val *pro-S* Me), 1.57 (3 H, s, 3_α-Me), 1.63 (3 H, s, 3_β-Me), 2.61 (1 H, d septet, *J* = 2.3, 7.0 Hz, C*H*Me$_2$), 3.90 (1 H, dd, *J* = 2.4, 1.5 Hz, 6-H), 4.00 (1 H, *J* = 11.7 Hz, 1_β-H), 4.30-4.35 (2 H, m, 1_α, 8a-H$_2$), 6.39 (1 H, br, 7-H); ^{13}C NMR ($CDCl_3$) (HMBC/HSQC) δ 15.88 (Val *pro-R* Me), 19.15 (Val *pro-S* Me), 23.68 (3_β-Me), 25.35 (3_α-Me), 28.12 (*C*HMe$_2$), 56.78 (8a-C), 60.57 (6-C), 65.40 (1-C), 96.05 (3-C), 163.12 (5-C), 168.38 (8-C); HRESIMS *m/z* 249.1202 $[M + Na]^+$ ($C_{11}H_{18}N_2NaO_3$requires 249.1215), 227.1370 $[M + H]^+$ ($C_{11}H_{19}N_2O_3$requires 227.1395).

6S,8aS-3,3-Dimethyl-6-(1-methylethyl) tetrahydrooxazolo[3,4-a]pyrazine-5,8-dione (5cS) Method B

Alloc-L-Leu-*S*-DmoOMe **8c*S*** was treated with $Pd(PPh_3)_4$ and DABCO, as for the synthesis of **5a*R*** (Method C), to give **5c*S*** (62%), with properties as above.

N-(N-(Prop-2-enyloxycarbonyl)-L-alanyl)-D-serine methyl ester (7dR)

Alloc-L-AlaOH **6a**[20] (1.00 g, 5.8 mmol) was stirred with HOBt (860 mg, 6.4 mmol) and DCC (1.31 g, 6.4 mmol) in

CH_2Cl_2 (20 mL) at 0°C for 1 h. D-Ser-OMe.HCl (820 mg, 5.8 mmol) and Et_3N (1.17 g, 11.6 mmol) were added and the mixture was stirred for 2 d at 20°C and allowed to stand at 4°C for 16 h. Filtration (Celite®), washing (aq. citric acid (10%), aq. $NaHCO_3$ (5%), water), drying, evaporation and chromatography (CH_2Cl_2/EtOAc 1:1) gave **7a*R*** (760 mg, 48%) as pale yellow crystals: mp 82–83°C. ^{1}H NMR ($CDCl_3$) δ 1.41 (3 H, dd, *J* = 6.9 Hz, Ala Me), 3.03 (1 H, br, OH), 3.77 (3 H, s, OMe), 3.95 (2 H, m, Ser β-H_2), 4.28 (1 H, qn, *J* = 6.9 Hz, Ala α-H), 4.56-4.61 (2 H, m, Alloc 1-H_2), 4.62 (1 H, dt, *J* = 7.7, 6.8 Hz, Ser α-H), 5.22 (1 H, ddd, *J* = 10.5, 3.0, 1.6 Hz, Alloc 3$_{cis}$-H), 5.30 (1 H, ddd, *J* = 18.8, 3.0, 1.6 Hz, Alloc 3$_{trans}$-H), 5.47 (1 H, br, Ala NH), 5.88 (1 H, m, Alloc 2-H), 7.13 (1 H, d, *J* = 7.2 Hz, Ser NH); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 18.57 (Ala Me), 50.96 (OMe), 52.87 (Ala α-C), 54.78 (Ser α-C), 62.82 (Ser β-C), 66.20 (Alloc 1-C), 118.21 (Alloc 3-C), 132.41 (Alloc 2-C), 156.23 (Alloc C=O), 170.95 (Ala C=O), 172.75 (Ser C=O).

*N-(N-(Prop-2-enyloxycarbonyl)-L-alanyl)-L-serine methyl ester (7a*S*)*

Alloc-L-AlaOH **6a**[20] was treated with L-SerOMe.HCl, HOBt and DCC, as for the synthesis of **7a*R***, to give **7a*S***[21] (44%) as pale yellow crystals: mp 86–87°C. ^{1}H NMR ($CDCl_3$) δ 1.39 (3 H, d, *J* = 7.2 Hz, Ala Me), 3.51 (1 H, br, OH), 3.76 (3 H, s, OMe), 3.92 (2 H, m, Ser β-H_2), 4.28 (1 H, qn, *J* = 7.2 Hz, Ala α-H), 4.53 (2 H, d, *J* = 4.1 Hz, Alloc 1-H_2), 4.63 (1 H, q, *J* = 7.4 Hz, Ser α-H), 5.19 (1 H, dd, *J* = 10.5, 1.4 Hz, Alloc 3$_{cis}$-H), 5.27 (1 H, ddd, *J* = 18.7, 3.0, 1.4 Hz, Alloc 3$_{trans}$-H), 5.64 (1 H, d, *J* = 7.4 Hz, Ala NH), 5.87 (1 H, m, Alloc 2-H), 7.16 (1 H, d, *J* = 7.4 Hz, Ser NH); ^{13}C-NMR ($CDCl_3$) (HSQC/HMBC) δ 18.57 (Ala Me), 50.50 (OMe), 52.83 (Ala α-C), 54.74 (Ser α-C), 62.58 (Ser β-C), 66.10 (Alloc 1-C), 118.10 (Alloc 3-C), 132.46 (Alloc 2-C), 156.25 (Alloc C=O), 170.95 (Ala C=O), 172.89 (Ser C=O).

*N-(N-(Prop-2-enyloxycarbonyl)-L-leucyl)-D-serine methyl ester (7b*R*)*

Alloc-L-LeuOH **6b**[20] was treated with D-SerOMe.HCl, HOBt and DCC, as for the synthesis of **7a*R***, to give **7b*R*** (92%) as a pale yellow gum. ^{1}H NMR ($CDCl_3$) δ 0.91 (3 H, d, *J* = 6.3 Hz, Leu Me), 0.94 (3 H, d, *J* = 6.3 Hz, Leu Me), 1.54 (1 H, dd, *J* = 9.4, 8.6 Hz, Leu β-H), 1.67 (2 H, m, Leu β, γ-H_2), 2.99 (1 H, br, OH), 3.76 (3 H, s, OMe), 3.93 (2 H, d, *J* = 3.6 Hz, Ser β-H_2), 4.21 (1 H, dd, *J* = 9.4, 3.6 Hz, Leu α-H), 4.55 (2 H, d, *J* = 5.5 Hz, Alloc 1-H_2), 4.61 (1 H, q, *J* = 7.4 Hz, Ser α-H), 5.20 (1 H, dd, *J* = 10.2, 1.4 Hz, Alloc 3$_{cis}$-H), 5.28 (1 H, ddd, *J* = 18.7, 2.7, 1.4 Hz, Alloc 3$_{trans}$-H), 5.34 (1 H, br, Leu NH), 5.88 (1 H, m, Alloc 2-H), 7.14 (1 H, d, *J* = 7.4 Hz, Ser NH); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 21.76 (Leu Me), 23.08 (Leu Me), 24.82 (Leu γ-C), 41.25 (Leu β-C), 52.86 (OMe), 53.73 (Leu α-C), 54.74 (Ser α-C), 62.63 (Ser β-C), 66.23 (Alloc 1-C), 118.19 (Alloc 3-C), 132.40 (Alloc 2-C), 156.39 (Alloc C=O), 170.94 (Leu C=O), 171.23 (Ser C=O).

*N-(N-(Prop-2-enyloxycarbonyl)-L-leucyl)-L-serine methyl ester (7b*S*)*

Alloc-L-LeuOH **6b**[20] was treated with L-SerOMe.HCl, HOBt and DCC, as for the synthesis of **7a*R***, to give **7b*S*** (55%) as a white powder: mp 82–83°C. ^{1}H NMR ($CDCl_3$) δ 0.92 (3 H, d, *J* = 6.3 Hz, Leu Me), 0.94 (3 H, d, *J* = 6.3 Hz, Leu Me), 1.54 (1 H, m, Leu β-H), 1.66–1.72 (2 H, m, Leu β,γ-H_2), 2.91 (1 H, t, *J* = 6.6 Hz, OH), 3.78 (3 H, s, OMe), 3.93-3.95 (2 H, m, Ser β-H_2), 4.16 (1 H, m, Leu α-H), 4.55 (2 H, d, *J* = 5.5 Hz, Alloc 1-H_2), 4.63 (1 H, q, *J* = 7.4 Hz, Ser α-H), 5.21 (1 H, dd, *J* = 10.5, 1.4 Hz, Alloc 3$_{cis}$-H), 5.29 (1 H, dd, *J* = 17.3, 1.4 Hz, Alloc 3$_{trans}$-H), 5.88 (1 H, m, Alloc 2-H), 6.86 (1 H, d, *J* = 7.4 Hz, Ser NH); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 22.02 (Leu Me), 22.94 (Leu Me), 24.73 (Leu γ-C), 41.28 (Leu β-C), 52.82 (OMe), 53.65 (Leu α-C), 54.74 (Ser α-C), 62.64 (Ser β-C), 66.16 (Alloc 1-C), 118.12 (Alloc 3-C), 132.44 (Alloc 2-C), 156.49 (Alloc C=O) 170.83 (Leu C=O), 172.61 (Ser C=O).

*N-(N-(Prop-2-enyloxycarbonyl)-L-valyl)-D-serine methyl ester (7c*R*)*

Alloc-L-ValOH **6c**[20] was treated with D-SerOMe.HCl, HOBt and DCC, as for the synthesis of **7a*R***, to give **7c*R*** (35%) as a white powder: mp 128–129°C. ^{1}H NMR ($CDCl_3$) δ 0.93 (3 H, d, *J* = 6.9 Hz, Val Me), 0.98 (3 H, d, *J* = 6.9 Hz, Val Me), 2.17 (1 H, m, Val β-H), 2.80 (1 H, t, *J* = 6.0 Hz, OH), 3.78 (3 H, s, OMe), 3.92-3.98 (2 H, m, Ser β-H_2), 4.04 (1 H, m, Val α-H), 4.56 (2 H, d, *J* = 5.5 Hz, Alloc 1-H_2), 4.64 (1 H, q, *J* = 6.9 Hz, Ser α-H), 5.21 (1 H, dd, *J* = 10.2, 1.4 Hz, Alloc 3$_{cis}$-H), 5.29 (1 H, dd, *J* = 17.4, 1.4 Hz, Alloc 3$_{trans}$-H), 5.38 (1 H, d, *J* = 7.4 Hz, Val NH), 5.90 (1 H, m, Alloc 2-H), 6.98 (1 H, d, *J* = 6.9 Hz, Ser NH); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 17.93 (Val Me), 19.32 (Val Me), 30.91 (Val β-C), 52.90 (OMe), 54.74 (Ser α-C), 60.57 (Val α-C), 63.0 0 (Ser β-C), 66.20 (Alloc 1-C), 118.19 (Alloc 3-C), 132.49 (Alloc 2-C), 156.64 (Alloc C=O), 170.87 (Val C=O), 171.59 (Ser C=O).

*N-(N-(Prop-2-enyloxycarbonyl)-L-valyl)-L-serine methyl ester (7c*S*)*

Alloc-L-ValOH **6c**[20] was treated with L-SerOMe.HCl, HOBt and DCC, as for the synthesis of **7a*R***, to give **7c*S*** (37%) as a white powder: mp 174–175°C. ^{1}H NMR ($CDCl_3$) δ 0.96 (3 H, d, *J* = 6.7 Hz, Val Me), 0.99 (3 H, d, *J* = 6.7 Hz, Val Me), 2.10 (1 H, m, Val β-H), 3.05 (1 H, br, OH), 3.78 (3 H, s, OMe), 3.95 (1 H, m, Val α-H), 3.97 (1 H, dd, *J* = 12.0, 6.6 Hz, Ser β-H), 4.00 (1 H, dd, *J* = 12.0, 6.6 Hz, Ser β-H), 4.55 (2 H, d, *J* = 5.5 Hz, Alloc 1-H_2), 4.67 (1 H, dt, *J* = 7.7, 6.6 Hz, Ser α-H), 5.21 (1 H, dd, *J* = 10.5, 1.4 Hz, Alloc 3$_{cis}$-H), 5.29 (1 H, dd, *J* = 17.0, 1.4 Hz, Alloc 3$_{trans}$-H), 5.42 (1 H, d, *J* = 8.0 Hz, Val NH), 5.82-5.96 (1 H, m, Alloc 2-H), 6.87 (1 H, d, *J* = 7.7 Hz, Ser NH); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 18.08 (Val Me), 19.24 (Val Me), 31.09 (Val β-C), 52.87 (OMe), 54.73 (Ser α-C), 60.77 (Val α-C), 62.90 (Ser β-C), 66.16 (Alloc 1-C), 118.11 (Alloc 3-C), 132.52 (Alloc 2-C), 156.57 (Alloc C=O), 170.75 (Val C=O), 171.71 (Ser C=O).

Methyl R-*2,2-dimethyl-3-((N-prop-2-enyloxycarbonyl)-L-alanyl)oxazolidine-4-carboxylate (8a*R*)*

Alloc-L-Ala-D-SerOMe **7a*R*** was treated with 2,2-dimethoxypropane and TsOH, as for the synthesis of **4a*R***, except that the solvent was toluene, the reaction time was 2 h and the chromatographic eluant was hexane/

EtOAc (7:3), to give **8a*R*** (79%) as a colourless oil: ^{1}H NMR ($CDCl_3$) δ 1.31 (3 H, d, *J* = 6.6 Hz, Ala Me), 1.54 (3 H, s, Dmo 2_α-Me), 1.64 (3 H, s, Dmo 2_β-Me), 3.79 (3 H, s, OMe), 4.14-4.18 (1 H, m, Ala α-H), 4.16 (1 H, dd, *J* = 9.4, 6.6 Hz, Dmo 5_α-H), 4.28 (1 H, d, *J* = 9.4 Hz, Dmo 5_β-H), 4.51 (2 H, t, *J* = 5.9 Hz, Alloc 1-H_2), 5.09 (1 H, d, *J* = 6.6 Hz, Dmo 4-H), 5.19 (1 H, dd, *J* = 10.5, 1.2 Hz, Alloc 3_{cis}-H), 5.26 (1 H, dd, *J* = 17.2, 1.2 Hz, Alloc 3_{trans}-H), 5.32 (1 H, d, *J* = 7.8 Hz, NH), 5.86 (1 H, m, Alloc 2-H); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 17.78 (Ala Me), 23.08 (Dmo 2_β-Me), 24.97 (Dmo 2_α-Me), 49.21 (Ala α-C), 52.88 (OMe), 59.35 (Dmo 4-C), 65.78 (Alloc 1-C), 66.96 (Dmo 5-C), 96.62 (Dmo 2-C), 117.69 (Alloc 3-C), 132.46 (Alloc 2-C), 155.90 (Alloc C=O), 170.41 (Ala C=O), 171.14 (Dmo C=O); HRESIMS *m/z* 337.1344 $[M + Na]^+$ ($C_{14}H_{22}N_2NaO_6$ requires 337.1375).

Methyl S-*2,2-dimethyl-3-((N-prop-2-enyloxycarbonyl)-L-alanyl)oxazolidine-4-carboxylate (8a*S*)*

Alloc-L-Ala-L-SerOMe **7a*S*** was treated with 2,2-dimethoxypropane and TsOH, as for the synthesis of **8a*R***, to give **8a*S***(82%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.31 (3 H, d, *J* = 6.6 Hz, Ala Me), 1.52 (3 H, s, Dmo 2_β-Me), 1.68 (3 H, s, Dmo 2_α-Me), 3.78 (3 H, s, OMe), 4.15 (1 H, dd, *J* = 9.4, 5.8 Hz, Dmo 5_β-H), 4.24 (1H, qn, *J* = 6.6 Hz, Ala α-H), 4.28 (1 H, dd, *J* = 9.4, 1.2 Hz, Dmo 5_α-H), 4.43 (1 H, d, *J* = 5.8 Hz, Dmo 4-H), 4.52 (2 H, dd, *J* = 5.2, 1.4 Hz, Alloc 1-H_2), 5.19 (1 H, dd, *J* = 10.4, 1.4 hz, Alloc 3_{cis}-H), 5.28 (1 H, dd, *J* = 17.0, 1.4 Hz, Alloc 3_{trans}-H), 5.62 (1 H, d, *J* = 7.8 Hz, NH), 5.89 (1 H, m, Alloc 2-H). ^{13}C NMR ($CDCl_3$) (HMBC/HSQC) δ 19.19 (Ala Me), 23.21 (Dmo 2_β-Me), 25.12 (Dmo 2_α-Me), 53.23 (OMe), 58.88 (Dmo 4-C), 60.37 (Ala α-C), 65.62 (Alloc 1-C), 66.99 (Dmo 5-C), 96.85 (Dmo 2-C), 117.58 (Alloc 3-C), 132.69 (Alloc 1-C), 155.09 (Alloc C=O), 169.72 (Ala C=O), 170.30 (Dmo C=O). HRESIMS *m/z* 337.1359 $[M + Na]^+$ ($C_{14}H_{22}N_2NaO_6$requires 337.1375).

Methyl R-*2,2-dimethyl-3-((N-prop-2-enyloxycarbonyl)-L-leucyl)oxazolidine-4-carboxylate (8b*R*)*

Alloc-L-Leu-D-SerOMe **7b*R*** was treated with 2,2-dimethoxypropane and TsOH, as for the synthesis of **8a*R***, to give **8b*R*** (78%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.82 (3 H, d, *J* = 6.6 Hz, Leu Me), 0.90 (3 H, d, *J* = 6.6 Hz, Leu Me), 1.43-1.50 (1 H, m, Leu β-H), 1.54 (3 H, s, Dmo 2_α-Me), 1.60 (2 H, m, Leu β,γ-H_2), 1.65 (3 H, s, Dmo 2_β-Me), 3.77 (3 H, s, OMe), 4.09 (1 H, m, Leu α-H), 4.18 (1 H, d, *J* = 5.9 Hz, Dmo 5_α-H), 4.23 (1 H, dd, *J* = 9.0, 1.6 Hz, Dmo 5_β-H), 4.52 (2 H, dd, *J* = 5.3, 1.6 Hz, Alloc 1-H_2), 5.10 (1 H, d, *J* = 5.9 Hz, Dmo 4-H), 5.19 (1 H, d, *J* = 10.5 Hz, Alloc 3_{cis}-H), 5.26 (1 H, dd, *J* = 17.2, 1.6 Hz, Alloc 3_{trans}-H), 5.87 (1 H, m, Alloc 2-H), 5.93 (1H, δ, *J* = 8.8 Hz, NH). ^{13}C NMR ($CDCl_3$) (HMBC/HSQC) d21.23 (Leu Me), 23.14 (Dmo 2_α-Me), 23.46 (Leu Me), 24.26 (Leu γ-H), 24.94 (Dmo 2_β-Me), 40.62 (Leu β-C), 51.93 (Leu α-C), 52.82 (OMe), 59.35 (Dmo 4-C), 65.85 (Alloc 1-C), 67.09 (Dmo 5-C), 96.66 (Dmo 2-C), 117.68 (Alloc 3-C), 132.51 (Alloc 2-H), 156.37 (Alloc C=O), 170.35 (Leu C=O), 171.15 (Dmo C=O). HRESIMS *m/z* 379.1816 $[M + Na]^+$ ($C_{17}H_{28}N_2NaO_6$requires 379.1840).

Methyl S-*2,2-dimethyl-3-((N-prop-2-enyloxycarbonyl)-L-leucyl)oxazolidine-4-carboxylate (8b*S*)*

Alloc-L-Leu-L-SerOMe**7b*S*** was treated with 2,2-dimethoxypropane and TsOH, as for the synthesis of **8a*R***, to give **8b*S*** (84%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.91 (3 H, d, *J* = 6.6 Hz, Leu Me), 0.94 (3 H, d, *J* = 6.3 Hz, Leu Me), 1.29 (1 H, m, Leu β-H), 1.48–1.53 (2 H, m, Leu β,γ-H_2), 1.51 (3 H, s, Dmo 2_β-Me), 1.68 (3 H, s, Dmo 2_α-Me), 3.79 (3 H, s, OMe), 4.17 (1 H, dd, *J* = 9.4, 6.3 Hz, Dmo 5_β-H), 4.24 (1 H, t, *J* = 9.5 Hz, Leu α-H), 4.25 (1 H, dd, *J* = 9.4, 1.6 Hz, Dmo 5_α-H), 4.42 (1 H, dd, *J* = 6.3, 1.6 Hz, Dmo 4-H), 4.51 (2 H, dd, *J* = 5.2, 1.4 Hz, Alloc 1-H_2), 5.17 (1 H, dd, *J* = 10.5, 1.4 Hz, Alloc 3_{cis}-H), 5.27 (1 H, dd, *J* = 17.0, 1.4 Hz, Alloc 3_{trans}-H), 5.37 (1 H, d, *J* = 9.1 Hz, NH), 5.88 (1 H, m, Alloc 2-H); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 21.69 (Leu Me), 23.33 (Dmo 2_β-Me), 23.41 (Leu Me), 24.45 (Leu γ-C), 25.01 (Dmo 2_α-Me), 43.17 (Leu β-C), 51.77 (Leu α-C), 53.25 (OMe), 58.92 (Dmo 4-C), 65.63 (Alloc 1-C), 66.96 (Dmo 5-C), 96.84 (Dmo 2-C), 117.51 (Alloc 3-C), 132.72 (Alloc 2-C), 155.59 (Alloc C=O), 170.35 (Leu C=O), 171.11 (Dmo C=O). HRESIMS *m/z* 379.1800 $[M + Na]^+$ ($C_{17}H_{28}N_2NaO_6$requires 379.1840).

Methyl R-*2,2-dimethyl-3-((N-prop-2-enyloxycarbonyl)-L-valyl)oxazolidine-4-carboxylate (8c*R*)*

Alloc-L-Val-D-SerOMe **7c*R*** was treated with 2,2-dimethoxypropane and TsOH, as for the synthesis of **8a*R***, to give **8c*R*** (86%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.91 (3 H, d, *J* = 6.9 Hz, Val Me), 0.94 (3 H, d, *J* = 6.9 Hz, Val Me), 1.55 (3 H, s, Dmo 2_α-Me), 1.68 (3 H, s, Dmo 2_β-Me), 2.02 (1 H, m, Val β-H), 3.78 (3 H, s, OMe), 3.83 (1 H, d, *J* = 8.8 Hz, Val α-H), 4.12 (1 H, dd, *J* = 9.4, 6.3 Hz, Dmo 5_α-H), 4.23 (1 H, d, *J* = 9.4 Hz, Dmo 5_β-H), 4.55 (2 H, dd, *J* = 5.5, 1.4 Hz, Alloc 1-H_2), 5.13 (2 H, m, Dmo 4-H and NH), 5.20 (1 H, dd, *J* = 10.5, 1.4 Hz, Alloc 3_{cis}-H), 5.27 (1 H, dd, *J* = 17.4, 1.4 Hz, Alloc 3_{trans}-H), 5.89 (1 H, m, Alloc 2-H); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 18.09 (Val Me), 19.67 (Val Me), 23.08 (Dmo 2_α-Me), 25.09 (Dmo 2_β-Me), 30.60 (Val β-C), 52.88 (OMe), 59.11 (Val α-C), 59.37 (Dmo 4-C), 66.00 (Alloc 1-C), 67.36 (Dmo 5-C), 96.84 (Dmo 2-C), 117.80 (Alloc 3-C), 132.56 (Alloc 2-C), 156.45 (Alloc C=O), 169.50 (Val C=O), 171.22 (Dmo C=O); HRESIMS *m/z* 341.1714 $[M - H]^-$ ($C_{16}H_{25}N_2O_6$requires 341.1712).

Methyl S-*2,2-dimethyl-3-((N-prop-2-enyloxycarbonyl)-L-valyl)oxazolidine-4-carboxylate (8c*S*)*

Alloc-L-Val-L-SerOMe **7c*S*** was treated with 2,2-dimethoxypropane and TsOH, as for the synthesis of **8a*R***, to give **8c*S*** (99%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.91 (3 H, d, *J* = 6.9 Hz, Val Me), 0.95 (3 H, d, *J* = 6.9 Hz, Val Me), 1.53 (3 H, s, Dmo 2_β-Me), 1.68 (3 H, s, Dmo 2_α-Me), 1.92 (1 H, octet, *J* = 6.9 Hz, Val β-H), 3.77 (3 H, s, OMe), 4.03 (1 H, dd, *J* = 9.4, 6.9 Hz, Val α-H), 4.17 (1 H, dd, *J* = 9.4, 6.3 Hz, Dmo 5_β-H), 4.26 (1 H, dd, *J* = 9.4, 1.7 Hz, Dmo 5_α-H), 4.52 (2 H, m, Alloc 1-H_2), 4.56 (1 H, dd, *J* = 6.3, 1.7 Hz, Dmo 4-H), 5.19 (1 H, dd, *J* = 10.5, 1.4 Hz, Alloc 3_{cis}-H), 5.28 (1 H, dd, *J* = 17.0, 1.4 Hz, Alloc 3_{trans}-H), 5.39 (1 H, d, *J* = 9.4 Hz, NH), 5.90 (1 H, m, Alloc 2-H); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 17.48 (Val Me), 19.13 (Val Me), 23.44 (Dmo 2_β-Me), 25.06 (Dmo 2_α-Me),

32.39 (Val β-C), 53.25 (OMe), 58.31 (Val α-H), 59.31 (Dmo 4-H), 65.66 (Alloc 1-C), 66.86 (Dmo 5-C), 96.85 (Dmo 2-C), 117.54 (Alloc 3-C), 132.78 (Alloc 2-C), 155.79 (Alloc C=O), 169.18 (Val C=O), 170.31 (Dmo C=O); HRESIMS *m/z* 365.1651 $[M + Na]^+$ ($C_{16}H_{26}N_2NaO_6$requires 365.1683).

R-2,2-Dimethyl-3-(N-prop-2-enyloxycarbonyl)-L-valinyl) tetrahydrooxazole-4-carboxylic acid (10R)

Alloc-L-Val-*R*-Dmo-OMe **4b*R*** (280 mg, 0.82 mmol) was stirred with aq. NaOH (5.0 M, 0.18 mL, 0.90 mmol) in MeOH (15 mL) for 16 h. The evaporation residue, in water, was acidified with citric acid (aq., 10%) to pH 5 and was extracted (EtOAc, 3×). Drying and evaporation gave **10*R*** (200 mg, 74%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.90 (3 H, d, *J* = 6.6 Hz, Val Me), 0.92 (3 H, d, *J* = 6.6 Hz, Val Me), 1.54 (3 H, s, 2-Me), 1.66 (3 H, s, 2-Me), 2.03 (1 H, m, Val β-H), 3.87 (1 H, t, *J* 9.0, Val γ-H), 4.12 (1 H, dd, *J* = 9.4, 6.6 Hz, 5-H), 4.32 (1 H, dd, *J* = 9.4, 5.1 Hz, 5-H), 4.47-4.57 (2 H, m, Alloc 1-H_2), 5.14-5.18 (2 H, m, 4-H and Alloc 3$_{cis}$-H), 5.24 (1 H, dd, *J* = 17.2, 1.6 Hz, Alloc 3$_{trans}$-H), 5.61 (1 H, d, *J* = 9.3 Hz, NH), 5.86 (1 H, m, Alloc 2-H), 7.25 (1 H, br, OH); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 18.13 (Val Me), 19.42 (Val Me), 22.91 (2-Me), 24.89 (2-Me), 30.43 (Val β-C), 59.31 (Val α-C), 59.40 (4-C), 65.86 (Alloc 1-C), 67.33 (5-C), 96.81 (2-C), 117.56 (Alloc 3-C), 132.48 (Alloc 2-C), 156.58 (Alloc C=O), 170.27 (Val C=O), 173.61 (Dmo C=O); HRESIMS *m/z* 655.3175 [2 *M* – H]$^-$ ($C_{30}H_{47}N_4O_{12}$requires 655.3203), 327.1571 [M – H]$^-$ ($C_{15}H_{23}N_2O_6$requires 327.1562).

S-2,2-Dimethyl-3-(N-prop-2-enyloxycarbonyl)-L-valinyl) tetrahydrooxazole-4-carboxylic acid (10S)

Alloc-L-Val-*S*-Dmo-OMe **4b*S*** was treated with NaOH, as for the synthesis of **10*R***, to give **10*S*** (85%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.87 (3 H, d, *J* = 6.6 Hz, Val Me), 0.93 (3 H, d, *J* = 6.6 Hz, Val Me), 1.49 (3 H, s, 2-Me), 1.63 (3 H, s, 2-Me), 1.89 (1 H, octet, *J* = 6.6 Hz, Val β-H), 4.09 (1 H, dd, *J* = 9.4, 6.6 Hz, 5-H), 4.27 (1 H, dd, *J* = 9.4, 6.6 Hz, Val α-H), 4.30 (1 H, d, *J* = 9.4 Hz, 5-H), 4.42 (1 H, d, *J* = 5.4 Hz, Alloc 1-H), 4.48 (1 H, d, *J* = 5.4 Hz, Alloc 1-H), 4.53 (1 H, d, *J* = 6.1 Hz, 4-H), 5.13 (1 H, dd, *J* = 10.5, 1.1 Hz, Alloc 3$_{cis}$-H), 5.23 (1 H, dd, *J* = 17.2, 1.1 Hz, Alloc 3$_{trans}$-H), 5.82 (1 H, m, Alloc 2-H), 5.94 (1 H, d, *J* = 9.4 Hz, NH), 7.25 (1 H, br, OH); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 17.30 (Val Me), 18.81 (Val Me), 23.31 (2-Me), 25.23 (2-Me), 32.63 (Val β-C), 57.97 (Val α-C), 59.11 (4-C), 66.29 (Alloc 1-C), 66.91 (5-C), 96.72 (2-C), 117.95 (Alloc 3-C), 132.25 (Alloc 2-C), 156.44 (Alloc C=O), 169.18 (Val C=O), 172.02 (Dmo C=O); HRESIMS *m/z* 655.3184 [2 *M* – H]$^-$ ($C_{30}H_{47}N_4O_{12}$requires 655.3203), 327.1565 [*M* – H]$^-$ ($C_{15}H_{23}N_2O_6$requires 327.1562).

Pentafluorophenyl R-2,2-dimethyl-3-(N-prop-2-enyloxycarbonyl)-L-valinyl)tetrahydrooxazole-4-carboxylate (11R)

Alloc-L-Val-*S*-Dmo-OH **10*R*** (210 mg, 0.64 mmol), pentafluorophenol (130 mg, 0.70 mmol) and DCC (140 mg, 0.70 mmol) were stirred in EtOAc (10 mL) at 0°C under N_2 for 4 h. Filtration (Celite®, twice) and evaporation gave **11*R*** (310 mg, quant.) as a pale yellow solid: mp 118–120°C; ^{1}H NMR ($CDCl_3$) δ 0.86 (3 H, d, *J* = 6.6 Hz, Val Me), 0.96 (3 H, d, *J* = 6.6 Hz, Val Me), 1.56 (3 H, s, 2-Me), 1.68 (3 H, s, 2-Me), 2.02 (1 H, m, Val β-H), 4.25-4.35 (2 H, m, Val α-H + 5-H), 4.40 (1 H, d, *J* = 9.9 Hz, 5-H), 4.54 (2 H, d, *J* = 5.5 Hz, Alloc 1-H_2), 5.19 (1 H, dd, *J* = 10.4, 1.1 Hz, Alloc 3$_{cis}$-H), 5.26 (1 H, dd, *J* = 17.1, 1.4 Hz, Alloc 3$_{trans}$-H), 5.50 (1 H, d, *J* = 9.1 Hz, NH), 5.66 (1 H, d, *J* = 6.0 Hz, 4-H), 5.87 (1 H, m, Alloc 2-H); ^{19}F NMR ($CDCl_3$) δ -161.37 (2 F, dd, *J* = 21.0, 17.1 Hz, 3',5'-F_2), −156.61 (1 F, t, *J* = 21.0 Hz, 4'-F), −152.68 (2 F, d, *J* = 17.1 Hz, 2',6'-F_2); HRESIMS *m/z* 517.1352 $[M + Na]^+$ ($C_{21}H_{23}F_5N_2NaO_6$requires 517.1373).

Pentafluorophenyl S-2,2-dimethyl-3-(N-prop-2-enyloxycarbonyl)-L-valinyl)tetrahydrooxazole-4-carboxylate (11S)

Alloc-L-Val-*S*-Dmo-OH **10*S*** was treated with pentafluorophenol and DCC, as for the synthesis of **11*R***, to give **11*S*** (quant.) as a pale yellow solid: mp 66–68°C; ^{1}H NMR ($CDCl_3$) δ 0.93 (3 H, d, *J* = 6.3 Hz, Val Me), 0.98 (3 H, d, *J* = 6.3 Hz, Val Me), 1.56 (3 H, s, 2-Me), 1.70 (3 H, s, 2-Me), 2.02 (1 H, m, Val β-H), 4.20 (1 H, dd, *J* = 9.0, 6.3 Hz, Val α-H), 4.27 (1 H, d, *J* = 6.6 Hz, 5-H), 4.44 (1 H, d, *J* = 6.6 Hz, 5-H), 4.50-4.58 (2 H, m, Alloc 1-H_2), 4.93 (1 H, d, *J* = 5.8 Hz, 4-H), 5.11 (1 H, d, *J* = 10.2 Hz, Alloc 3$_{cis}$-H), 5.27 (1 H, d, *J* = 17.2 Hz, Alloc 3$_{trans}$-H), 5.46 (1 H, *J* = 9.3 Hz, NH), 5.83 (1 H, m, Alloc 2-H); ^{19}F NMR ($CDCl_3$) δ –161.88 (2 F, dd, *J* = 21.0, 17.1 Hz, 3',5'-F_2), −157.27 (1 F, t, *J* = 21.0 Hz, 4'-F), −152.36 (2 F, d, *J* = 17.1 Hz, 2',6'-F_2); HRESIMS *m/z* 517.1385 $[M + Na]^+$ ($C_{21}H_{23}F_5N_2NaO_6$requires 517.1373).

R-2,2-Dimethyl-3-(N-allyloxycarbonyl)-L-valinyl)-N-(2-(4-chlorophenyl)ethyl)tetrahydrooxazole-4-carboxamide (12R)

2-(4-Chlorophenyl)ethylamine hydrochloride (117 mg, 0.61 mmol) was stirred with Et_3N (122 mg, 1.2 mmol) and **7b*R*** (300 mg, 0.61 mmol) in CH_2Cl_2 (4.0 mL) for 2 h. Evaporation and chromatography (EtOAc/hexane 3:7) gave **12*R*** (280 mg, 70%) as a colourless gum: ^{1}H NMR ($CDCl_3$) δ 0.79 (3 H, d, *J* = 6.5 Hz, Val Me), 0.87 (3 H, d, *J* = 6.7 Hz, Val Me), 1.47 (3 H, s, 2-Me), 1.58 (3 H, s, 2-Me), 1.93 (1 H, m, Val β-H), 2.76 (2 H, m, $ArCH_2$), 3.40 (1 H, dq, *J* = 13.4, 6.7 Hz, $ArCH_2CH$), 3.53 (1 H, dq, *J* = 13.4, 6.7 Hz, $ArCH_2CH$), 3.86 (1 H, t, *J* = 7.6 Hz, Val α-H), 3.99-4.08 (2 H, m, 5-H_2), 4.48-4.53 (2 H, m, Alloc 1-H_2), 4.82 (1 H, d, *J* = 5.8 Hz, 4-H), 5.14 (1 H, d, *J* = 10.5 Hz, Alloc 3$_{cis}$-H), 5.21 (1 H, d, *J* = 17.3 Hz, Alloc 3$_{trans}$-H), 5.56 (1 H, br, Val NH), 5.82 (1 H, m, Alloc 2-H), 6.47 (1 H, br, CH_2NH), 7.07 (2 H, d, *J* = 8.2 Hz, Ar 2,6-H_2), 7.20 (2 H, d, *J* = 8.2 Hz, Ar 3,5-H_2); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 17.82 (Val Me), 19.38 (Val Me), 20.86 (Val β-C), 22.68 (2-Me), 24.99 (2-Me), 34.69 ($ArCH_2CH_2$), 40.54 ($ArCH_2$), 59.03 (Val α-C), 60.23 (5-C), 60.40 (4-C), 65.73 (Alloc 1-C), 96.43 (2-C), 117.56 (Alloc 3-C), 128.66 (Ar 3,5-C_2), 129.92 (Ar 2,6-C_2), 132.37 (Alloc 2-C and Ar 4-C), 136.72 (Ar 1-C), 156.40 (Alloc C=O), 169.96 (Val C=O), 170.98 (Dmo C=O); HRESIMS *m/z* 488.1924 $[M + Na]^+$ ($C_{23}H_{32}{}^{35}ClN_3NaO_5$ requires 488.1922), 466.2116 $[M + H]^+$ ($C_{23}H_{33}{}^{35}ClN_3O_5$ requires 466.2103).

S-2,2-Dimethyl-3-(N-allyloxycarbonyl)-L-valinyl)-N-(2-(4-chlorophenyl)ethyl)tetrahydrooxazole-4-carboxamide (12S)

Compound **7b*S*** was treated with 2-(4-chlorophenyl) ethylamine hydrochloride, as for the synthesis of **12*R***

except that the chromatographic eluant was EtOAc/hexane (2:3), to give **12*S*** (81%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.89 (3 H, d, *J* = 6.5 Hz, Val Me), 0.94 (3 H, d, *J* = 6.5 Hz, Val Me), 1.44 (3 H, s, 2-Me), 1.45 (3 H, s, 2-Me), 1.86 (1 H, m, Val β-H), 2.76 (2 H, t, *J* = 7.1 Hz, $ArCH_2$), 3.42 (1 H, m, $ArCH_2CH$), 3.51 (1 H, m, $ArCH_2CH$), 3.76 (1 H, t, *J* = 6.5 Hz, Val α-H), 3.99 (1 H, t, *J* = 7.0 Hz, 5-H), 4.28 (1 H, d, *J* = 5.8 Hz, 4-H), 4.34 (1 H, d, *J* = 9.1 Hz, 5-H), 4.41-4.51 (2 H, m, Alloc 1-H_2), 5.15 (1 H, d, *J* = 10.2 Hz, Alloc 3$_{cis}$-H), 5.24 (1 H, d, *J* = 17.2 Hz, Alloc 3$_{trans}$-H), 5.26 (1 H, d, *J* = 6.8 Hz, Val NH), 5.82 (1 H, m, Alloc 2-H), 7.05 (2 H, d, *J* = 8.2 Hz, Ar 2,6-H_2), 7.15 (2 H, d, *J* = 8.2 Hz, Ar 3,5-H_2), 7.27 (1 H, br, CH_2NH); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 17.34 (Val Me), 19.41 (Val Me), 22.46 (2-Me), 25.67 (2-Me), 30.62 (Val β-C), 34.11 ($ArCH_2CH_2$), 40.86 ($ArCH_2$), 58.74 (Val α-C), 60.22 (4-C), 65.91 (Alloc 1-C), 67.63 (5-C), 96.83 (2-C), 117.93 (Alloc 3-C), 128.49 (Ar 3,5-C_2), 130.03 (Ar 2,6-C_2), 132.04 (Alloc 2-C), 132.33 (Ar 4-C), 137.25 (Ar 1-C), 156.55 (Dmo C=O), 169.01 (Val C=O), 169.32 (alloc C=O); HRESIMS *m/z* 488.1890 $[M + Na]^+$ ($C_{23}H_{32}{}^{35}ClN_3NaO_5$ requires 488.1922), 466.2084 $[M + H]^+$ ($C_{23}H_{33}{}^{35}ClN_3O_5$ requires 466.2103).

R-*(2,2-Dimethyl-3-L-valinyl)-N-(2-(4-chlorophenyl)ethyl) tetrahydrooxazole-4-carboxamide(13*R*)*

Compound **12*R*** (5.0 mg, 10 mmol) was stirred with $Pd(PPh_3)_4$ (1.0 mg, 9 mmol) and Me_3SiNMe_2 (9.0 mg, 77 mmol) in CH_2Cl_2 (1.0 mL) and water (0.5 mL). After 2 h, the reaction was quenched with aq. $NaHCO_3$ (5.0 mL) and the mixture was extracted with CH_2Cl_2 (2 × 10 mL). After drying and evaporation, the crude material was analysed by NMR in $CDCl_3$ (0.6 mL): ^{1}H NMR ($CDCl_3$) δ 0.75 (3 H, d, *J* = 6.6 Hz, Val Me), 0.90 (3 H, d, *J* = 6.6 Hz, Val Me), 1.25 (3 H, s, 2-Me), 1.54 (3 H, s, 2-Me), 1.86 (1 H, d septet, *J* = 8.6, 6.6 Hz, Val β-H), 2.77 (1 H, d, *J* = 8.6 Hz, Val α-H), 2.80 (2 H, m, $ArCH_2$), 3.39 (1 H, dq, *J* = 13.2, 6.6 Hz, $ArCH_2CH$), 3.68 (1 H, dq, *J* = 13.2, 6.6 Hz, $ArCH_2CH$), 4.08 (1 H, d, *J* = 9.6 Hz, 5$_α$-H), 4.15 (1 H, dd, *J* = 9.6, 6.0 Hz, 5$_β$-H), 4.57 (1 H, d, *J* = 6.0 Hz, 4-H), 6.00 (1 H, br, NH), 7.09 (2 H, d, *J* = 8.2 Hz, Ph-H_2), 7.26 (2 H, d, *J* = 8.2 Hz, Ph-H_2); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 17.59 (Val Me), 20.09 (Val Me), 26.25 (2-Me), 29.68 (2-Me), 31.85 (Val β-C), 34.65 ($ArCH_2CH_2$), 40.38 ($ArCH_2$), 60.87 (4-C and Val α-C), 67.66 (5-C), 96.20 (C_q), 128.94 (2 × Ph-C), 129.97 (2 × Ph-C), 132.13 (Ph-C_q), 136.39 (Ph-C_q), 171.47 (C=O), 172.53 (C=O). HRESIMS *m/z* 404.1704 $[M + Na]^+$ ($C_{19}H_{28}{}^{35}ClN_3NaO_3$ requires 404.1716), 382.1894 $[M + H]^+$ ($C_{19}H_{29}{}^{35}ClN_3O_3$ requires 382.1897).

S-*(2,2-Dimethyl-3-L-valinyl)-N-(2-(4-chlorophenyl)ethyl) tetrahydrooxazole-4-carboxamide(13*S*)*

Compound **12*S*** was treated with $Pd(PPh_3)_4$ and Me_3SiNMe_2, as for the synthesis of **13*R***, to give crude **13*S***: ^{1}H NMR ($CDCl_3$) δ 0.82 (3 H, d, *J* = 6.6 Hz, Val Me), 0.85 (3 H, d, *J* = 6.6 Hz, Val Me), 1.47 (3 H, s, 2-Me), 1.49 (3 H, s, 2-Me), 1.51 (2 H, br, NH_2), 1.75 (1 H, d septet, *J* = 8.6, 6.6 Hz, Val β-H), 2.72-2.80 (3 H, m, $ArCH_2$ + Val α-H), 3.47 (1 H, dq, *J* = 12.4, 6.2 Hz, $ArCH_2CH$), 3.55 (1 H, dq, *J* = 12.4, 6.2 Hz, $ArCH_2CH$), 4.02 (1 H, dd, *J* = 9.3, 6.7 Hz, 5-H), 4.29 (2 H, m, 4-H + 5-H), 6.85 (1 H, br, NH), 7.06 (2 H, d, *J* = 8.6 Hz, Ph-H_2), 7.19 (2 H, d, *J* = 8.6 Hz, Ph-H_2); ^{13}C NMR ($CDCl_3$) (HSQC/HMBC) δ 17.59 (Val Me), 19.51 (Val Me), 22.54 (2-Me), 25.90 (2-Me), 32.85 (Val β-C), 34.45 ($ArCH_2CH_2$), 40.86 ($ArCH_2$), 60.20 (Val α-C), 60.83 (4-C), 67.25 (5-C), 96.54 (C_q), 128.78 (2 × Ph-C), 130.15 (2 × Ph-C), 132.03 (Ph-C_q), 132.12 (Ph-C_q), 170.10 (C=O), 172.52 (C=O); HRESIMS *m/z* 382.1892 $[M + H]^+$ ($C_{19}H_{29}{}^{35}ClN_3O_3$ requires 382.1897).

X-ray Crystallography

Single crystals of compounds **5a*R***, **5b*R*** and **5c*R*** were analysed at 150 (2) K using graphite-monochromated Mo $K_α$ radiation (λ = 0.71073 Å) and a Nonius Kappa CCD diffractometer. Details of the data collections, solutions and refinements are given in **Table 1**. The structures were uniformly solved using SHELXS-97 and refined using full-matrix least squares in SHELXL-97.[22,23] Crystallographic data for **5a*R***, **5b*R*,** and **5c*R*** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications CCDC 767300, CCDC 767301, and CCDC 767302, respectively. Requests for data should be addressed to CCDC, 12 Union Road, CambridgeCB2 1EZ, UK.

MTS assay. The cytotoxicity of **5b*R*** was assayed using HT29 human colon carcinoma cells in an MTS assay, using the method previously described by us.[24]

CONCLUSION

In this paper, we have reported the synthesis of two diastereomeric series of *cyclo*-L-aminoacyl-*R*/*S*-Dmo (DKPs). The conformations in solution in deuterochloroform were studied by NMR; for the L,*R* series, solid-state conformations were also studied by X-ray crystallography. The L,*S* series had the DKP in a boat conformation with the oxazolidine in a half-chair; the L,*R* series had the DKP in a flattened conformation with the oxazolidine in an alternative half-chair. The conformations are very similar in each case to those of the corresponding Dmt-containing DKPs reported previously.[2] The potential utility of L,L-DKPs lacking the second (oxazolidine or thiazolidine) ring, as rigid three-dimensional scaffolds has been reviewed.[25] Moreover, a natural product, aspergilazine A, which contains a Pro-DKP unit, has been shown to have useful anti-influenza A (H_1N_1) activity;[26] understanding the conformations of Pro-DKPs may shed light on mechanisms of action and aid further drug design. Early kinetic studies have shown that L,*R*-Dmo dipeptide amides cyclised more rapidly than L,*S*-diastereoisomers, expelling the model drug and that the rate of cyclisation in the L,*R* series depends inversely on steric bulk at the α-carbon. We have previously reported on the mechanistic basis of this effect in the Dmt analogues,[2] which is due to steric clashes as the nucleophilic amine approaches the amide carbonyl to form the first tetrahedral intermediate. A similar mechanistic rationale is likely here in the Dmo series. One example, *cyclo*-L-Leu-*R*-Dmo **5b*R***, has been shown to be completely devoid of toxicity to HT29 human

Table 1: Crystal data and structure refinement of 5a*R*, 5b*R*, and 5c*R*

Compound	5a*R*	5b*R*	5c*R*
Empirical formula	$C_9H_{14}N_2O_3$	$C_{12}H_{20}N_2O_3$	$C_{11}H_{18}N_2O_3$
Formula weight	198.22	240.30	226.27
Temperature	150 (2) K	150 (2) K	150 (2) K
Wavelength	0.71073 Å	0.71073 Å	0.71073 Å
Crystal system	Monoclinic	Orthorhombic	Orthorhombic
Space group	P21	P212121	P212121
a/Å	5.5770 (2)	6.5510 (1)	7.2820 (4)
b/Å	5.8500 (2)	10.5190 (2)	7.5770 (4)
c/Å	14.9810 (6)	18.5310 (4)	21.4560 (14)
α/°	90	90	90
β/°	93.002 (1)	90	90
γ/°	90	90	90
U/Å^3	488.09 (3)	1276.97 (4)	1183.85 (12)
Z	2	4	4
Do/g.cm^{-3}	1.349	1.250	1.270
M/mm^{-1}	0.102	0.090	0.093
F (000)	212	520	488
Crystal size	0.40×0.30×0.07 mm	0.25×0.25×0.06 mm	0.31×0.18×0.07 mm
Theta min, max/°	3.66, 27.57	3.66, 27.45	3.88, 27.54
Index ranges	$-7\leq h\leq 7;-7\leq k\leq 7;-19\leq l\leq 19$	$-8\leq h\leq 8;-13\leq k\leq 13;-24\leq l\leq 24$	$-8\leq h\leq 8;-9\leq k\leq 9;26\leq l\leq 27$
Reflections collected	8785	23605	8269
Independent reflections, R (int)	2217, 0.0467	2920, 0.0630	2502, 0.0536
Reflections observed (>2σ)	2094	2491	2014
Data Completeness	0.996	0.997	0.940
Data/restraints/parameters	2217/2/136	2920/1/163	2502/1/154
Goodness-of-fit on F^2	1.050	1.038	1.043
Final R1, wR2 indices [I>2(I)]	0.0319, 0.0786	0.0352, 0.0714	0.0442, 0.0979
Final R1, wR2indices (all data)	0.0345, 0.0804	0.0504, 0.0765	0.0677, 0.1088
Flack parameter	0.2 (9)	−0.1 (10)	−0.5 (14)
Largest diff. peak and hole/eÅ^{-3}	0.200, −0.141	0.216, −0.222	0.226, −0.253

cells, indicating that its generation during the release of active drugs from prodrugs should be safe.

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