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Synthesis of pyrazolo[1,2-*a*]pyrazole-based peptide mimetics

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Dedicated to Professor Emeritus Volker Jäger, Institut für Organische Chemie, Universität Stuttgart, on the Occasion of his 70th Anniversary

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ABSTRACT

The synthesis of U-shaped conformationally constrained analogues of peptides based on the 3-amino-2-oxo-1,5-diazabicyclo[3.3.0]octane-8-carboxylic acid scaffold was developed. [3+2] Cycloadditions of (1*Z*,4*R**,5*R**)-1-arylmethylidene-4-benzyloxycarbonylamino-3-oxo-5-phenylpyrazolidin-1-ium-2-ides to *tert*-butyl acrylate and *tert*-butyl methacrylate gave the corresponding racemic cycloadducts, in most cases as mixtures of isomers, which were separated by preparative chromatography. Selective deprotection of the C- and the N-terminal of these heterocyclic dipeptides followed by coupling with (*S*)-alanine derivatives and chromatographic separation furnished the non-racemic tripeptides as target compounds. The structures of racemic cycloadducts and non-racemic final products were determined by NMR spectroscopy and X-ray diffraction. The synthesized compounds were also evaluated for inhibition of MurD ligase and D-alanine:D-alanine ligase.

1. Introduction

The synthesis of molecules that can mimic the structure and, hence, the properties of peptides certainly represent an interesting and important research topic in the fields of organic synthesis and medicinal chemistry. Within this context, replacement of a dipeptide motif in a given native (or natural) substrate with a U-shaped conformationally constrained heterocyclic analogue that simulates the β-turn structure, is of particular interest.[1] An important group of conformationally constrained dipeptide analogues are azabicycloalkane amino acids, comprising various saturated 4,5-fused, 5,5-fused, 5,6-fused, and related fused heterocycles with a bridgehead nitrogen atom.[2] 3-Amino-2-oxo-1,5-diazabicyclo[3.3.0]octane-8-carboxylic acid (or 6-amino-7-oxotetrahydropyrazolo[1,2-*a*]pyrazole-1-carboxylic acid) (**1**) based scaffolds are a subgroup of 5,5-fused azabicycloalkane amino acids. The most general and straightforward synthetic approach towards derivatives of **1** includes stereoselective 1,3-dipolar cycloaddition as the key-step (Fig. 1). Generally, derivatives of **1** are prepared from 4-acylamino-3-pyrazolidinones as starting compounds, which in turn are easily available by simple treatment of α,β-dehydro-α-amino acid derivatives with excess hydrazine hydrate.[3,8] Retrosynthetic analysis of derivatives of **1** (cf. Fig. 1) also reveals that compounds with up to five stereogenic centres are available in four steps and from four building blocks: (a) *N*-acylglycines, (b) aldehydes (or ketones) (c) hydrazine hydrate, and (d) α,β-unsaturated esters. Thus, combination of just four types of widely available building blocks enables an easy access to structurally diverse heterocyclic dipeptides (Fig. 1).

The importance of pyrazolidin-3-one derivatives has grown increasingly over the last decades due to their synthetic applicability and biological activity.[3] Recent applications of 3-pyrazolidinones include their use as templates in enantioselective Diels–Alder,[4] Michael,[5] and 'click' reactions,[6] while Eli Lilly's antibiotics are typical examples of bioactive pyrazolo[1,2-*a*]pyrazolone derivatives.[7] Thus, the applicability of 3-amino-2-oxo-1,5-diazabicyclo[3.3.0]octane-7-carboxylic acid (**1**) for the preparation of biologically active peptide mimetics has been successfully demonstrated by Eli Lilly's researchers almost three decades ago,[7] however, a literature survey reveals very few other examples of **1**-derived compounds since 1990. For example, a substructure

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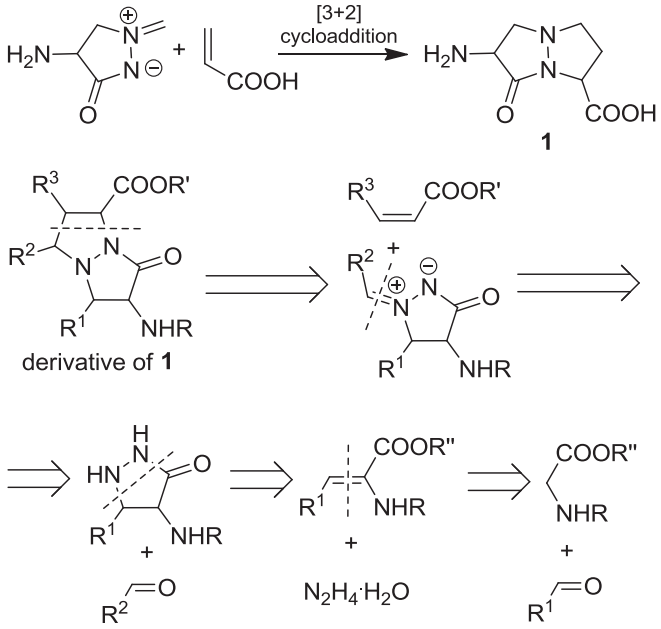


Fig. 1. 3-Amino-2-oxo-1,4-diazabicyclo[3.3.0]octane-7-carboxylic acid (**1**) as scaffold for preparation of peptide mimetics and retrosynthetic analysis of derivatives of **1**.

search on **1** with locked ring fusion gives only one hit, i.e., Eli Lilly's patent from 1989.[7e] Thus, **1** is a practically unexplored scaffold that should have reasonable applicative potential.

1,3-Dipolar cycloadditions provide an easy access to polyfunctional five-membered heterocycles with multiple stereogenic centres, usually with excellent stereocontrol.[9] Asymmetric cycloadditions are well elaborated with chiral nitrones, nitrile oxide, and azomethine ylide series,[10] however, far fewer examples of cycloadditions to chiral azomethine imines have been reported.[11] In the last decade, our studies on [3+2] cycloadditions of (1*Z*,4*R**,5*R**)-1-arylmethylidene-4-benzoylamino-3-oxo-5-phenyltetrahydropyrazol-1-ium-2-ides (1-arylmethylidene-4-benzoylamino-5-phenylpyrazolidin-3-on-1-azomethine imines) to various dipolarophiles has revealed general reactivity and selectivity of these cycloadditions,[8] as well as their applicability in high-throughput synthesis.[12]

Though very useful for the determination of reactivity and selectivity of the above [3+2] cycloadditions, the obtained cycloadducts were not suitable for incorporation into peptides, since carboxy and amino functions (COOMe and NHCOPh, respectively) could not be selectively deprotected without cleaving the pyrazolo[1,2-*a*]pyrazolone system as well. Thus, selective deprotection of a heterocyclic dipeptide was mandatory for a viable method for the synthesis of peptide mimetics. To do this, we decided to try out a classical peptide chemistry approach utilizing a combination of Boc and Cbz protecting groups. Cycloadditions of 1-arylmethylidene-4-benzyloxycarbonylamino-3-oxopyrazolidin-1-azomethine imines to *tert*-butyl acrylate (or some other *tert*-butyl 2-alkenoate) would give selectively deprotectable dipeptides enabling derivatization of the carboxy and the amino function. Furthermore, coupling of the racemic dipeptide with an enantiomerically pure reagent (e.g., with α-amino acid derivative), followed by separation of the so formed diastereomers would give non-racemic tripeptides.[13]

Herein, we report the results of this study, i.e., the synthesis of *tert*-butyl 3-benzyloxycarbonylamino-2-oxo-1,4-diazabicyclo-[3.3.0]octane-7-carboxylates as heterocyclic peptides with variable amino acid sequence and configuration and their use as building blocks in the synthesis of **1**-based conformationally constrained peptide mimetics.

2. Results and discussion

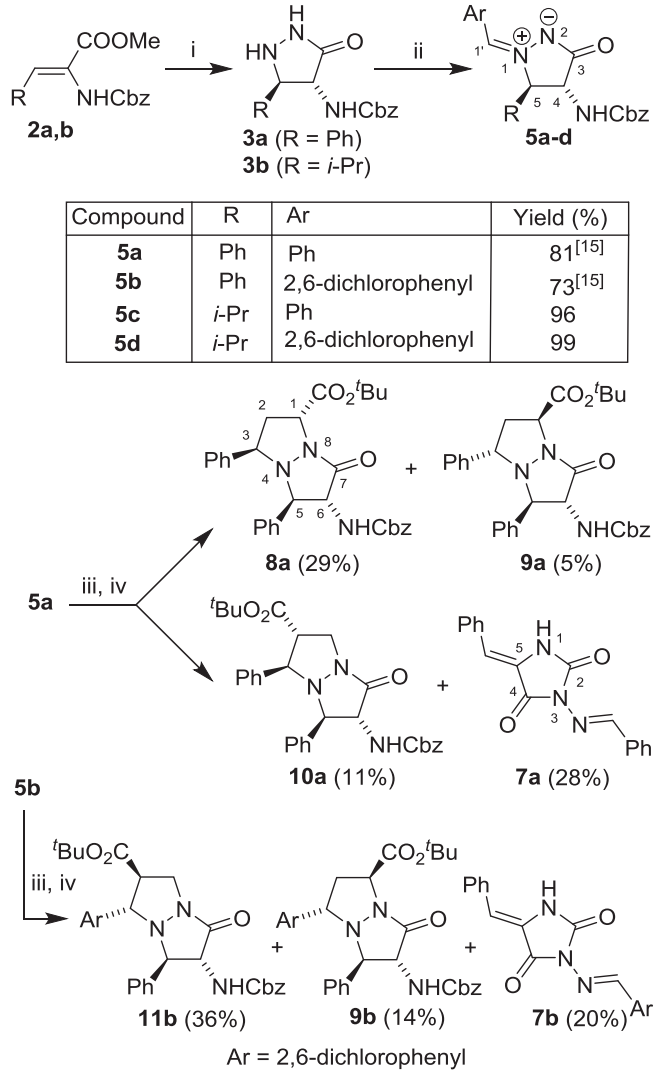
2.1. Synthesis of racemic pyrazolo[1,2-*a*]pyrazolone-based dipeptide building blocks 8–23

The starting model pyrazolidinones **3a** (R=Ph) and **3b** (R=*i*-Pr) were prepared by treatment of α,β-dehydro-α-amino acid derivatives **2a**,**b**[14] with hydrazine hydrate following the literature procedure.[15] Further acid-catalysed treatment of **3a**,**b** with benzaldehyde (**4a**) and 2,6-dichlorobenzaldehyde (**4b**) gave the *ortho*-unsubstituted azomethine imines **5a**,**c** and their *ortho*-disubstituted analogues **5b**,**d** in 73–99% yields.[15] First, cycloadditions of 5-phenyl substituted dipoles **5a**,**b** to *tert*-butyl acrylate (**6a**) were carried out under standard conditions, i.e., in refluxing anisole.[8] Somewhat unexpectedly,[15] reactions of 5-phenyl substituted dipoles **5a**,**b** furnished, along with cycloadducts **8**–**11**, the hydantoin derivatives **7a**,**b** in ~25% yields. As shown previously,[15] formation of **7a**,**b** is explainable by a 'ring switching' transformation consisting of thermal cleavage of the benzylic C(5)–N(1) bond followed by condensation between the amidic nitrogen N(2) and the benzyloxycarbonyl group. Compounds **7a**,**b** were obtained as precipitates and were removed by filtration. Evaporation of the filtrates followed by separation by column chromatography (CC) and medium pressure liquid chromatography (MPLC) then furnished the corresponding cycloadducts **8a**–**10a**, **9b** and **11b** in 5–36% yields (Scheme 1).

On the other hand, the C(5)–N(1) bond in 5-isopropyl substituted dipoles **5c**,**d** were thermally stable and formation of hydantoins **7** was not observed in cycloadditions of azomethine imines **5c**,**d** to *tert*-butyl acrylate (**6a**), which produced only the corresponding cycloadducts **8**–**12**. Subsequent chromatographic separation of isomeric cycloadducts furnished isomerically pure compounds **8c**, **10c** and **9d**–**12d** in 7–33% yields (Scheme 2).

In contrast, cycloadditions of **5c**,**d** to *tert*-butyl methacrylate (**6b**) were regio- and stereo-selective. Cycloaddition of **6b** to dipole **5c** followed by chromatographic separation furnished diastereomeric cycloadducts **13c** and **14c** in 35% and 9% yield, respectively, while cycloaddition of **6b** to the *ortho*-disubstituted dipole **5d** gave compound **14d** as the only product in 66% yield (Scheme 3).

The regioselectivity and stereoselectivity of the cycloadditions and configurations of the major isomers **8a**, **8c**, **11b**, **13c** and **14d** were in agreement with previous results obtained by cycloadditions of closely analogous dipoles to methyl acrylate[16] and methyl methacrylate.[17] The major 1-CO_2^tBu regioisomers **8a**, **8c** and **13c** obtained from *ortho*-unsubstituted dipoles **5a** and **5c** exhibited *syn*-orientation between the substituents at positions 3 and 5 and, vice versa, the major 2-CO_2^tBu regioisomers **11b**, **11d** and **14d** obtained from *ortho*-disubstituted dipoles **5b** and **5d** exhibited *anti*-orientation of the substituents at positions 3 and 5. The *trans*-configuration of the substituents at positions 2 and 3 in the 2-COOBu-*t* regioisomers **10a**, **10c**, **10d**, **11b** and **11d** was also in agreement with closely related literature examples.[16] Selectivity of these cycloadditions was somewhat lower than expected on the basis of results with analogous reactions.[8,12,16,17] Nevertheless, isolation of multiple isomers may also be advantageous, due to the increase of the stereochemical diversity of 6-amino-7-oxotetrahydropyrazolo[1,2-*a*]pyrazole-1(or 2)-carboxylic acid scaffold (cf. Schemes 1–3). Having this small, stereochemically diverse library of dipeptides **8**–**14** in our hands, we continued with selective deprotection of the amino and the carboxy function. Acidolytic deprotection of the carboxy group of dipeptides **8a**, **8c**, **9b**, **11b**, **11d**, **13c** and **14d** afforded the corresponding carboxylic acids **15**–**19** in 67–100% yields, while hydrogenolytic deprotection of the amino group of **8a**, **8c**, **11b**, **11d**, **13c** and **14d** afforded the corresponding free amines **20**–**23** in 20–94% yields. According to



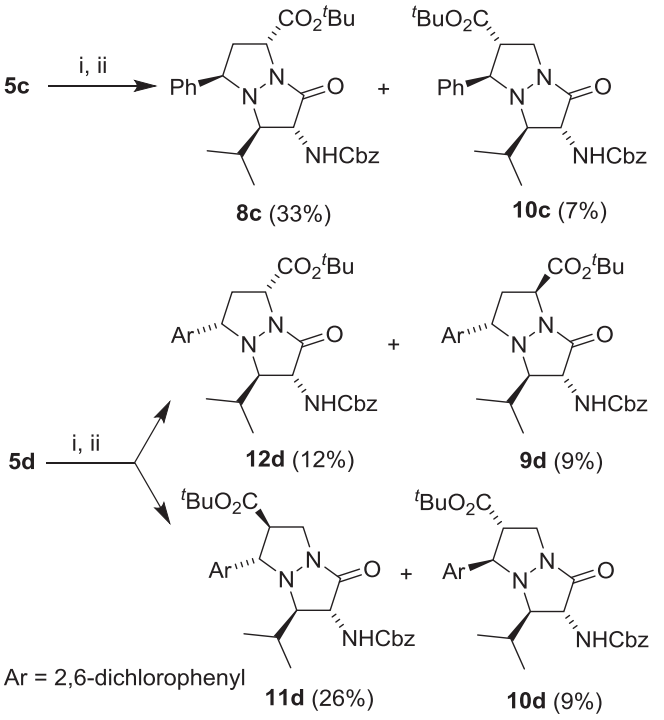
Compound	R	Ar	Yield (%)
5a	Ph	Ph	81[15]
5b	Ph	2,6-dichlorophenyl	73[15]
5c	*i*-Pr	Ph	96
5d	*i*-Pr	2,6-dichlorophenyl	99

Scheme 1. Reaction conditions: (i) hydrazine hydrate, MeOH, rt (Ref. 15); (ii) ArCHO (**4a**,**b**), EtOH, TFA (cat.), rt (Ref. 15); (iii) *tert*-butyl acrylate (**6a**), anisole, reflux; (iv) chromatographic separation (CC, MPLC).

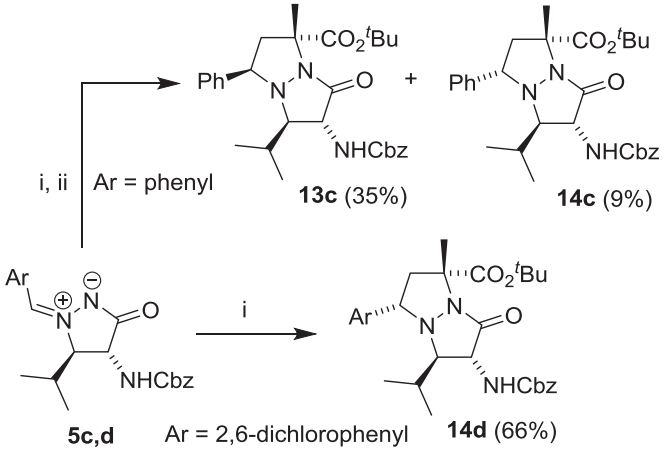
the previously reported results,[15] the low yield of compound **20a** could be explained by partial hydrogenolytic cleavage of the C(5)–N(1) bond, which induces further transformations of the primary product **20a** (Scheme 4).

2.2. Synthesis of racemic tetrapeptide 28 and hexapeptide 33

To show that compounds **15**–**23** can serve as useful building blocks for the synthesis of U-shaped peptides, tetrapeptide **28** and hexapeptide **33** with 3-amino-2-oxo-1,4-diazabicyclo[3.3.0]octane-7-carboxylic acid (**1**) as the central part of the sequence were prepared. Amidation of dipeptide **18c** with glycine methyl ester (**24**) gave the tripeptide **25** in 50% yield. Catalytic hydrogenation of **25** followed by acylation of **26** with Boc-glycine (**27**) in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) furnished tetrapeptide **28** in 87% yield. Similarly, acylation of **22c** with *N*-(*N*-benzyloxycarbonylglycyl)glycine (**29**) gave the tetrapeptide **30** in 74% yield. Treatment of **30** with trifluoroacetic acid in dichloromethane afforded the carboxylic acid **31** in 23% yield. Amidation of the acid **31** with *N*-(glycyl)glycine methyl ester (**32**) furnished the hexapeptide **33** in 62% yield (Scheme 5).



Scheme 2. Reaction conditions: (i) *tert*-butyl acrylate (**6a**), anisole, reflux; (ii) chromatographic separation (CC, MPLC).

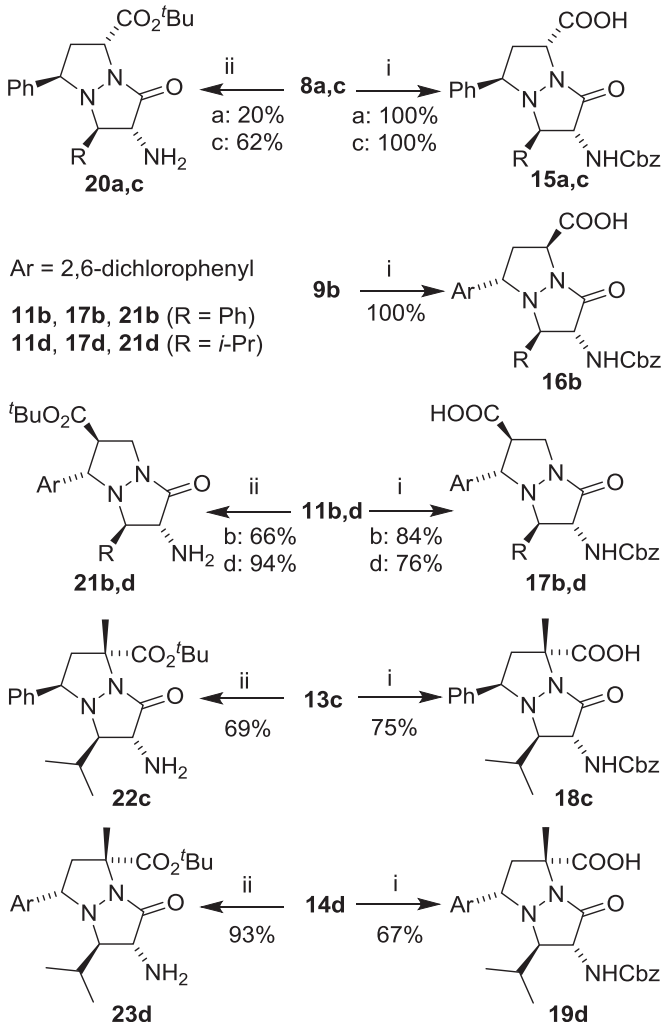


Scheme 3. Reaction conditions: (i) *tert*-butyl methacrylate (**6b**), anisole, reflux; (ii) chromatographic separation (CC, MPLC).

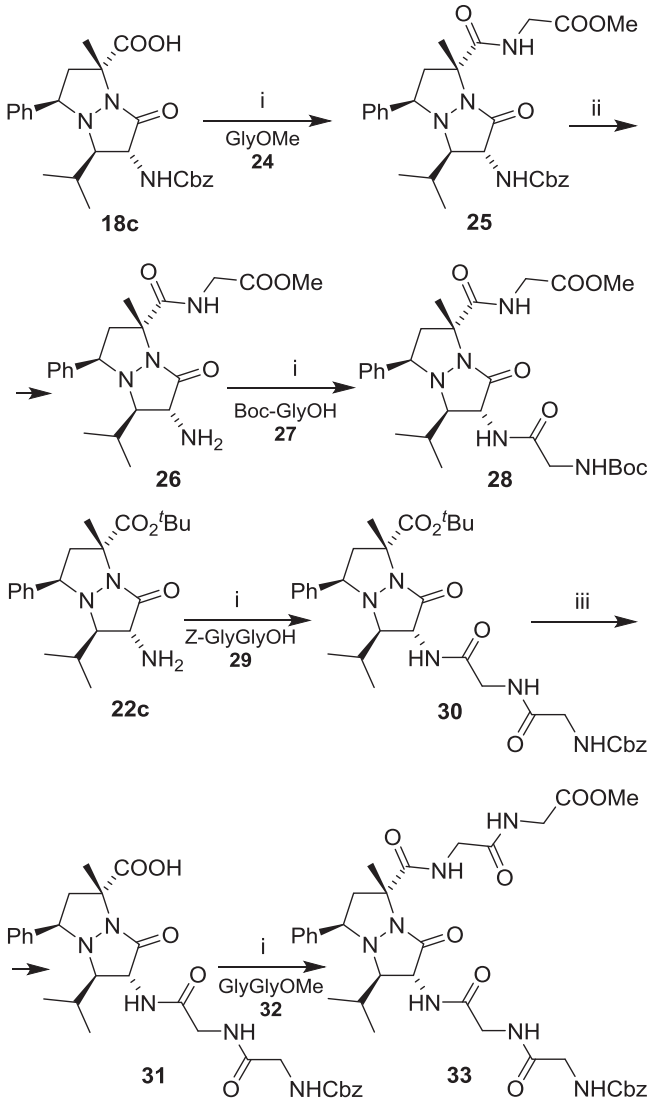
2.3. Synthesis of non-racemic pyrazolo[1,2-*a*]pyrazolone-based tripeptides 35–42 and 35′–42′

Finally, sixteen non-racemic tripeptides were prepared. The first example was amidation of the racemic *N*-protected dipeptides **15a**,**c** and **18c** with methyl (*S*)-alaninate (**34**) giving diastereomeric tripeptides **35**/**35′** and **36**/**36′**, which were separated by MPLC to furnish the non-racemic, diastereomerically pure *N*-protected tripeptide esters **35a**,**c**, **35a**,**c′**, **36c** and **36c′**. Similarly, acylation of the racemic *C*-protected dipeptide **20c** and **22c** with (*S*)-Boc-alanine (**37**) in the presence of EEDQ followed by separation of diastereomers by MPLC afforded the non-racemic tripeptide esters **38c**, **38c′**, **39c** and **39c′** (Scheme 6).

In the same manner, racemic carboxylic acids **16b** and **19d** were transformed with methyl (*S*)-alaninate (**34**) into mixtures of diastereomeric tripeptides **40b**/**40b′** and **41d**/**41d′**, while acylation of the racemic amine **23d** with Boc-(*S*)-alanine afforded a mixture of



Scheme 4. Reaction conditions: (i) CH_2Cl_2–CF_3COOH (2:1), rt; (ii) H_2, Pd–C, EtOH, rt.



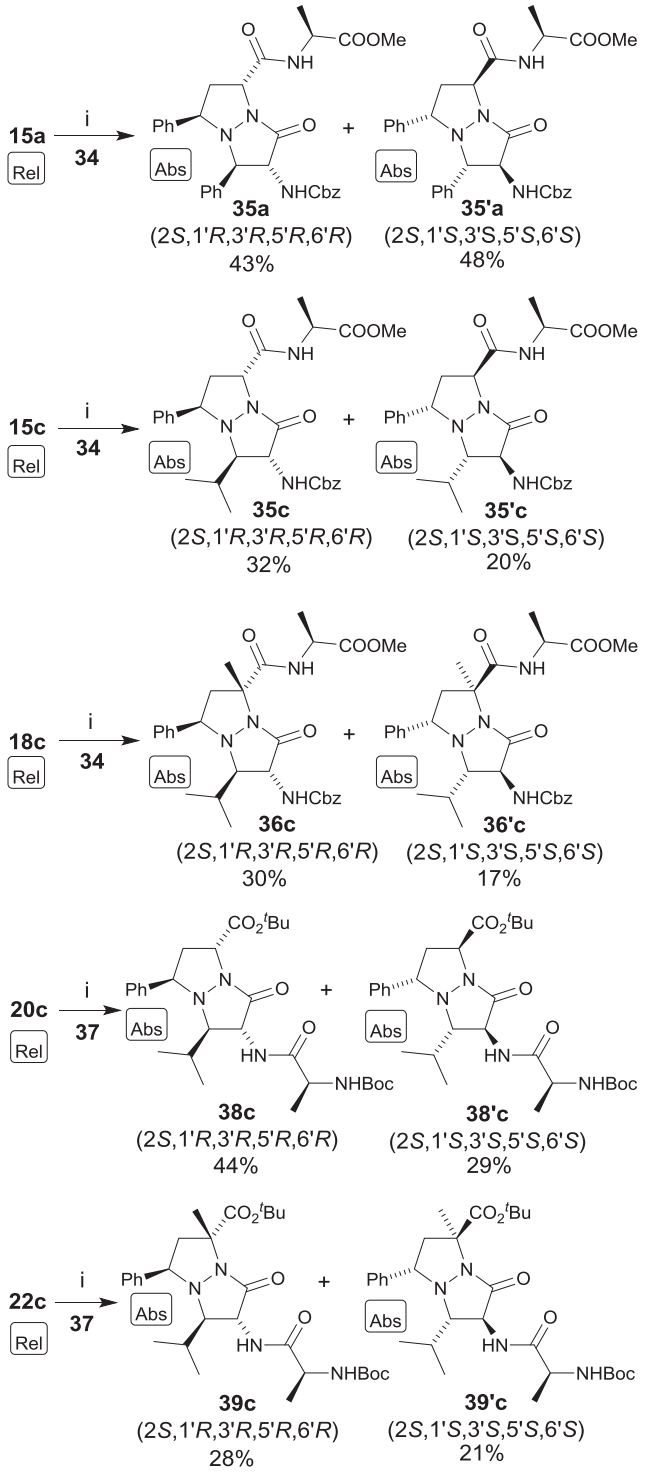
Scheme 5. Reaction conditions: (i) EEDQ, CH_2Cl_2, rt; (ii) H_2, Pd–C, EtOH, rt; (iii) CH_2Cl_2–CF_3COOH (2:1), rt.

diastereomeric tripeptides **42d**/**42d′**. Separation of the mixtures of diastereomers by MPLC furnished isomerically pure non-racemic tripeptide esters **40b**, **40b′**, **41d**, **41d′**, **42d** and **42d′** (Scheme 7).

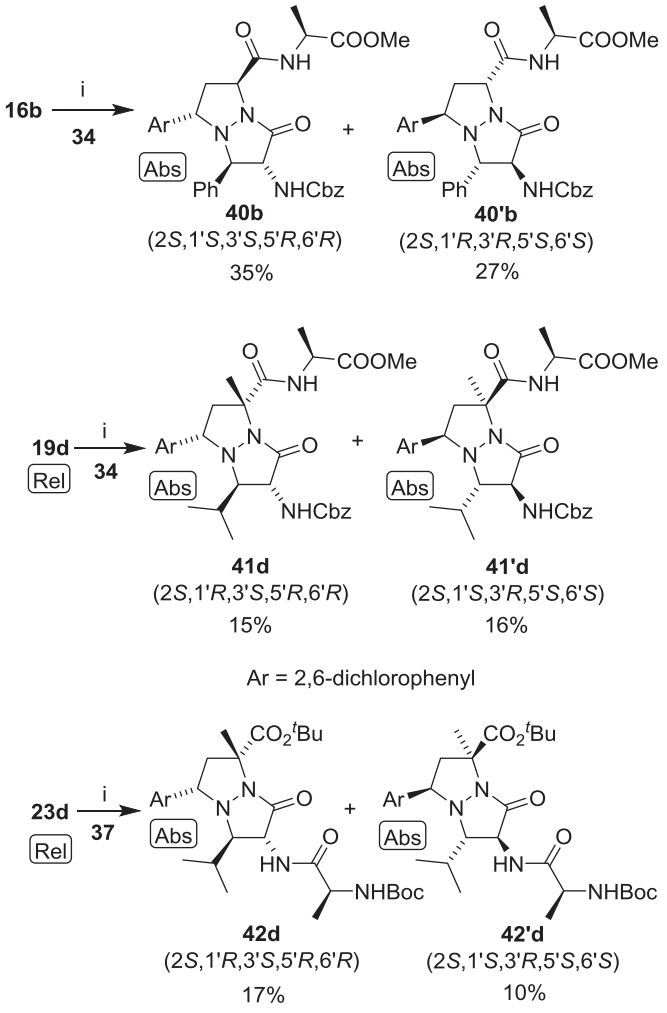
2.4. Structure determination

The structures of novel compounds **3b**, **5c**,**d**, **8**–**23**, **25**, **26**, **28**, **30**, **31**, **35**/**35′**, **36**/**36′**, and **38**/**38′**–**42**/**42′** were determined by spectroscopic methods (^{1}H NMR, ^{13}C NMR, 2D NMR, NOESY spectroscopy, HRMS, IR) and by analyses for C, H, and N. Spectral data for compounds **3b**, **5c**,**d**, **8**–**23**, **25**, **26**, **28**, **30**, **31**, **35**/**35′**, **36**/**36′**, and **38**/**38′**–**42**/**42′** were in agreement with the data for closely related pyrazolidinone derivatives.[8,12,13,16–18] The configuration of the pyrazolo[1,2-*a*]pyrazole core in compounds **8**–**23**, **25**, **26**, **28**, **30**, **33** and **35**/**35′**–**42**/**42′** was determined by NMR. Detailed structure determination by NMR spectroscopy is given in the Supplementary data. Finally, the structures of compounds **5c**, **5d**, **9d**, **11b**, **11d**, **21b**, **36c′**, **39c′** and **40b′** were determined by X-ray diffraction (Figs. 2–10). X-ray structures of dipoles **5c** and **5d** (cf. Figs. 2 and 3) are, to the best of our knowledge, the first examples in the (1*Z*,4*R**,5*R**)-1-arylmethylidene-4-acylamino-3-oxo-5-phenyltetrahydropyrazol-1-ium-2-ide series, which unambiguously proved the Z-configuration around the exocyclic C=N double bond.

Since the absolute configurations of the non-racemic tripeptides **36c′**, **39c′** and **40b′** were unambiguously established by X-ray diffraction, the configurations of their diastereomers **36c**, **39c** and **40b** were determined unambiguously as well. For the other 3,5-*syn*-diastereomers **35a**, **35a′**, **35c**, **35c′**, **38c** and **38c′**, the tentative configurations were assigned on the basis of correlation between specific rotation and absolute configuration of diastereomeric tripeptides **36c**, **36c′**, **39c** and **39c′**. Thus, the tentative (2*S*,1′*R*,3′*R*,5′*R*,6′*R*)-configuration was assigned to diastereomers **35a**, **35c** and **38c** exhibiting strong negative specific rotations, while tentative (2*S*,1′*S*,3′*S*,5′*S*,6′*S*)-configuration was assigned to diastereomers **35a′**, **35c′**, and **38c′** with strong positive specific rotations. Such correlation was not clearly applicable for the 3,5-*anti*-diastereomers **40**/**40′**–**42**/**42′**, since the diastereomers **40b** and **40b′** with unambiguously determined absolute configuration exhibited only a weak specific rotation, positive for the (2*S*,1′*S*,3′*S*,5′*R*,6′*R*)-isomer **40b** and negative for the (2*S*,1′*R*,3′*R*,5′*S*,6′*S*)-isomer **40b′**. This 'inversion' of specific rotation in **40b**/**40b′** with respect to specific rotation pattern observed in compounds **35**/**35′**, **36**/**36′**, **38**/**38′**, and **39**/**39′** coincides with opposite configurations at positions 1′ and 3′. On the other hand, other 3,5-*anti*-diastereomers **41d**/**41d′** and **42d**/**42d′** are 3′-epimers of **36c**/**36c′** and **39**/**39c′** and, most probably, the sign of specific rotation could not be changed upon reversal of configuration at



Scheme 6. Reaction conditions: (i) methyl (*S*)-alaninate (**34**) or Boc-(*S*)-alanine (**37**), EEDQ, CH_2Cl_2, rt, then chromatographic separation (FC, MPLC).



Scheme 7. Reaction conditions: (i) methyl (*S*)-alaninate (**34**) or Boc-(*S*)-alanine (**37**), EEDQ, CH_2Cl_2, rt, then chromatographic separation (FC, MPLC).

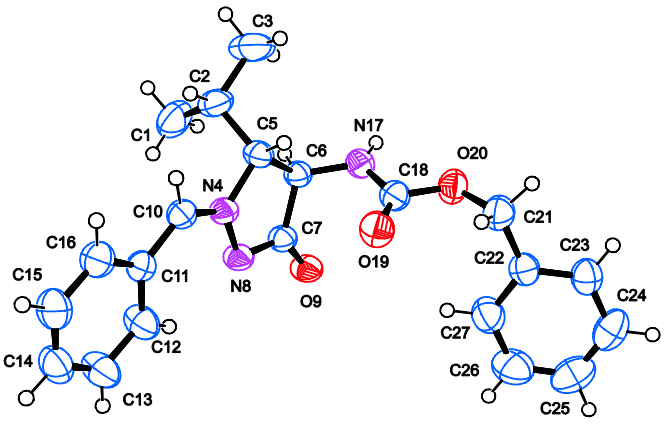


Fig. 2. The molecular structure of **5c**, showing the atom labelling. The displacement ellipsoids are drawn with 50% probability and the hydrogen atoms are shown as small spheres of arbitrary radii.

only one stereogenic centre (among five). Therefore, correlation of specific rotation of the 3,5-*anti*-diastereomers **41d**/**41d**′ and **42d**/**42d**′ with epimeric compounds **35**/**35**′, **36**/**36**′, **38**/**38**′ and **39**/**39**′ seems quite reasonable. Accordingly, the tentative (2*S*,1′*R*,3′*S*,5′*R*,6′*R*)-configuration was proposed for the isomers **41d** and **42d** with a strong negative specific rotation and the tentative (2*S*,1′*S*,3′*R*,5′*S*,6′*S*)-configuration for the isomers **41d**′ and **42d**′ with a strong positive specific rotation (Table 1, cf. Schemes 6 and 7).

The X-ray structures of dipeptides **11b**, **11d** and **21b** and tripeptides **36c**′, **39c**′ and **40b**′ exhibit the U-shaped structure of the peptide chain (cf. Figs. 5–10). The U-shape of **36c**′ is additionally stabilized by intramolecular N–H···O=C hydrogen bond (with N13···O9 distance of 2.808(3) Å) donated by N13 from the alanyl residue and accepted by O9 of the C=O group. The hydrogen bond is represented as a dashed line in Fig. 8.

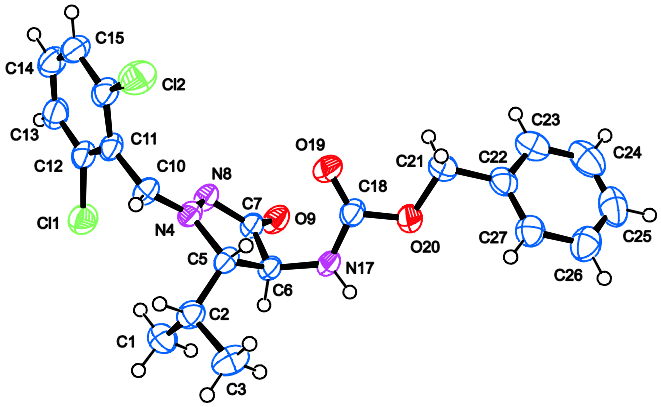


Fig. 3. The molecular structure of **5d**, showing the atom labelling. The displacement ellipsoids are drawn with 50% probability and the hydrogen atoms are shown as small spheres of arbitrary radii.

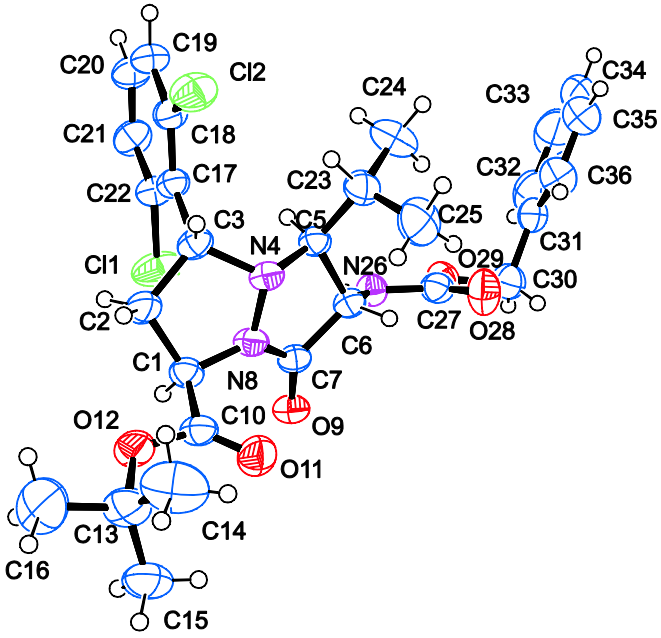


Fig. 4. The molecular structure of **9d**, showing the atom labelling. The displacement ellipsoids are drawn with 50% probability and the hydrogen atoms are shown as small spheres of arbitrary radii.

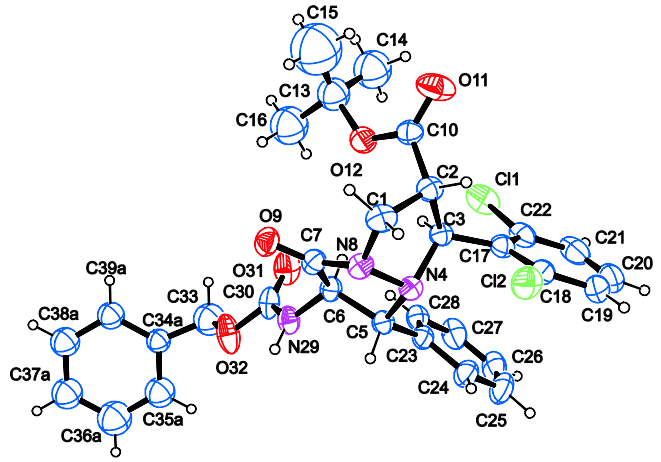


Fig. 5. The molecular structure of **11b**, showing the atom labelling. The displacement ellipsoids are drawn with 50% probability and the hydrogen atoms are shown as small spheres of arbitrary radii.

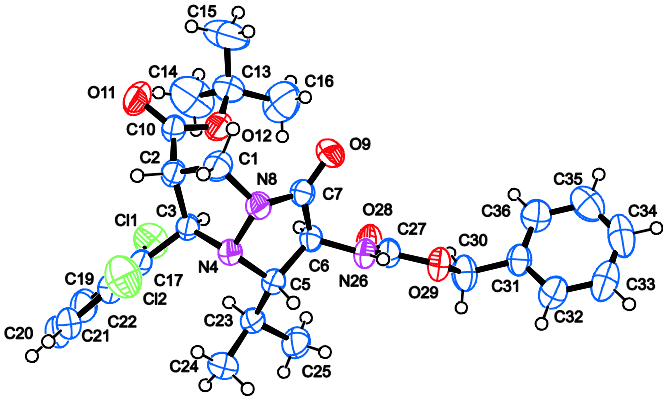


Fig. 6. The molecular structure of **11d**, showing the atom labelling. The displacement ellipsoids are drawn with 50% probability and the hydrogen atoms are shown as small spheres of arbitrary radii.

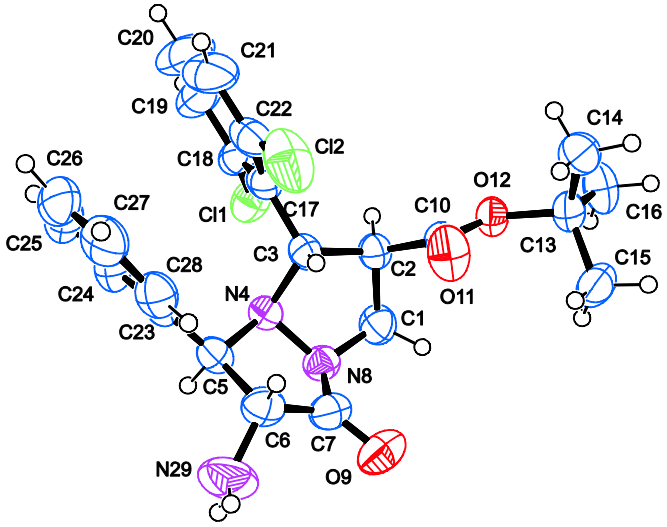


Fig. 7. The molecular structure of **21b**, showing the atom labelling. The displacement ellipsoids are drawn with 50% probability and the hydrogen atoms are shown as small spheres of arbitrary radii.

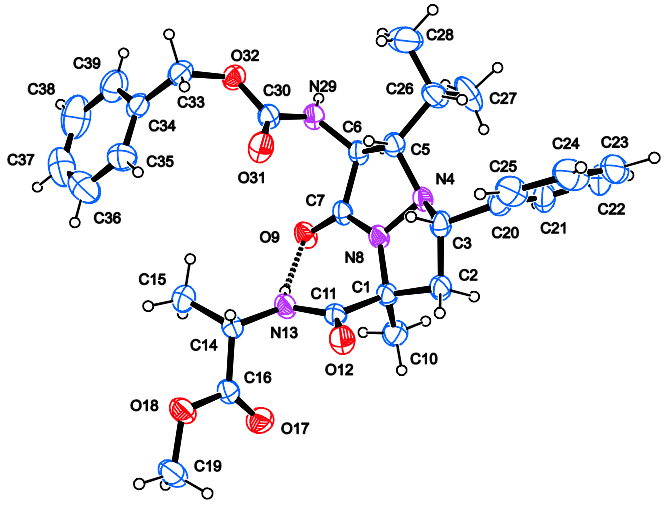


Fig. 8. The molecular structure of **36c′**, showing the atom labelling. The displacement ellipsoids are drawn with 50% probability and the hydrogen atoms are shown as small spheres of arbitrary radii. The dashed line represents the intramolecular hydrogen bond N13–H13···O9 that stabilizes U-shape of the molecule.

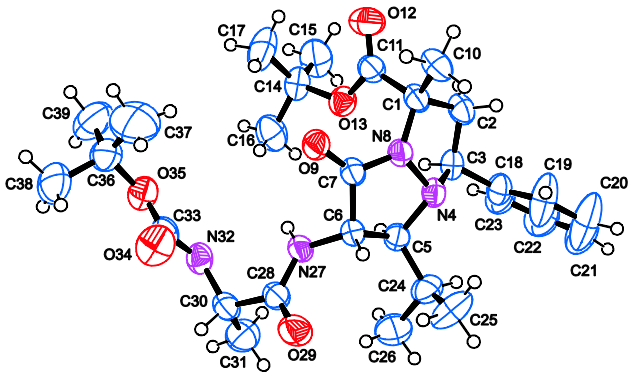


Fig. 9. The molecular structure of **39c′**, showing the atom labelling. The displacement ellipsoids are drawn with 50% probability and the hydrogen atoms are shown as small spheres of arbitrary radii.

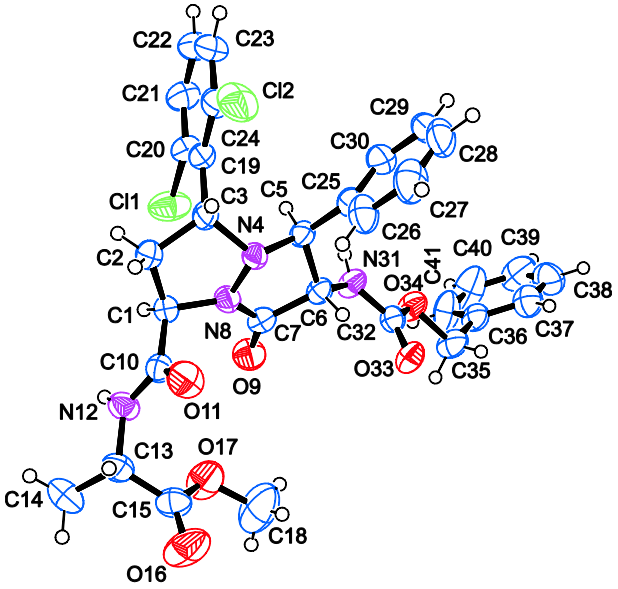


Fig. 10. The molecular structure of **40b′**, showing the atom labelling. The displacement ellipsoids are drawn with 50% probability and the hydrogen atoms are shown as small spheres of arbitrary radii.

Table 1
Correlation between specific rotation and absolute configuration of the non-racemic diastereomeric pairs of tripeptides **35**/**35′**, **36**/**36′**, **38**/**38′**, **39**/**39′** and **40**/**40′**–**42**/**42′**

Entry	Isomer	Configuration	$[\alpha]_D^{25}$
1[b]	**35a**	2*S*,1′*R*,3′*R*,5′*R*,6′*R*[b]	−74.3 (*c* 0.15, EtOH)
2[b]	**35a′**	2*S*,1′*S*,3′*S*,5′*S*,6′*S*[b]	+62.7 (*c* 0.15, EtOH)
3[b]	**35c**	2*S*,1′*R*,3′*R*,5′*R*,6′*R*[b]	−33.0 (*c* 0.12, $CDCl_3$)
4[b]	**35c′**	2*S*,1′*S*,3′*S*,5′*S*,6′*S*[b]	+82.9 (*c* 0.11, $CDCl_3$)
5	**36c**	2*S*,1′*R*,3′*R*,5′*R*,6′*R*	−108 (*c* 0.12, EtOH)
6[a]	**36c′**	2*S*,1′*S*,3′*S*,5′*S*,6′*S*[a]	+81.7 (*c* 0.16, EtOH)
7[b]	**38c**	2*S*,1′*R*,3′*R*,5′*R*,6′*R*[b]	−61.6 (*c* 0.13, $CDCl_3$)
8[b]	**38c′**	2*S*,1′*S*,3′*S*,5′*S*,6′*S*[b]	+62.6 (*c* 0.12, $CDCl_3$)
9	**39c**	2*S*,1′*R*,3′*R*,5′*R*,6′*R*	−66.2 (*c* 0.13, EtOH)
10[a]	**39c′**	2*S*,1′*S*,3′*S*,5′*S*,6′*S*[a]	+52.7 (*c* 0.11, EtOH)
11	**40b**	2*S*,1′*S*,3′*S*,5′*R*,6′*R*	+6.9 (*c* 0.12, EtOH)
12[a]	**40b′**	2*S*,1′*R*,3′*R*,5′*S*,6′*S*[a]	−18.2 (*c* 0.21, EtOH)
13[b]	**41d**	2*S*,1′*R*,3′*S*,5′*R*,6′*R*[b]	−128 (*c* 0.10, EtOH)
14[b]	**41d′**	2*S*,1′*S*,3′*R*,5′*S*,6′*S*[b]	+106 (*c* 0.11, EtOH)
15[b]	**42d**	2*S*,1′*R*,3′*S*,5′*R*,6′*R*[b]	−93.9 (*c* 0.41, EtOH)
16[b]	**42d′**	2*S*,1′*S*,3′*R*,5′*S*,6′*S*[b]	+72.7 (*c* 0.17, EtOH)

[a] Determined by X-ray diffraction.
[b] Tentative configuration.

In $CDCl_3$ solution, formation of (7′)C=*O*···*H*–N–C(2) intramolecular hydrogen bond in tripeptides **35**/**35′**, **36**/**36′**, **40**/**40′** and **41**/**41′** with the *C*-terminal (*S*)-alanyl residue was supported by ^{1}H NMR spectroscopy. Typically, the signals for the (non-hydrogen bonded) amidic NH protons in tripeptides **35**/**35′**, **36**/**36′** and **38**/**38′**–**42**/**42′** appeared at a chemical shift of δ=5–7 ppm, while the signals for the hydrogen bonded 2-NH protons exhibited higher chemical shift, δ=7.5–9.3 ppm. For example, in the ^{1}H NMR spectrum of tripeptide **36c′** with the *C*-terminal (*S*)-alanine residue, a doublet for the H–N–C(2) proton at 8.39 ppm indicates non-covalent interactions of this NH group, explainable by (7′)C=*O*···*H*–N–C(2) intramolecular hydrogen bond. In contrast, a broad singlet for 2-NH proton at 4.94 ppm in the ^{1}H NMR spectrum of tripeptide **39c′** with the *N*-terminal (*S*)-alanine residue does not support hydrogen bonding of this NH group (Fig. 11, Table 2).

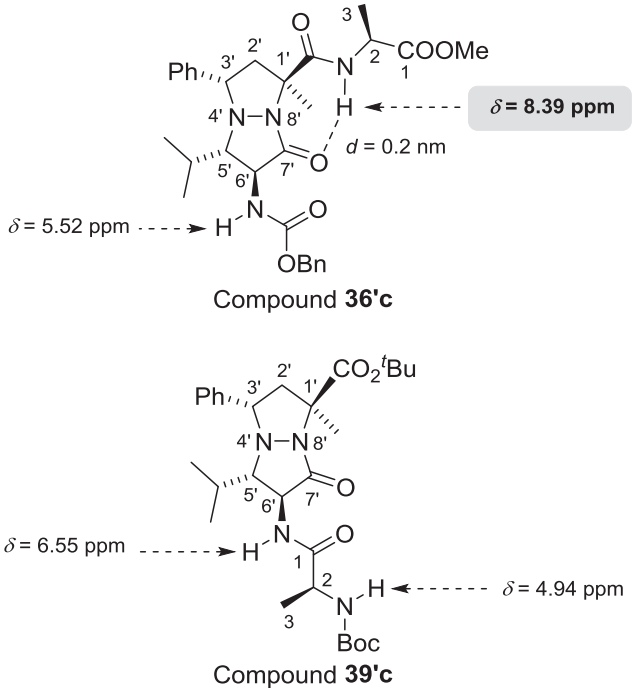


Fig. 11. Chemical shifts of the NH protons in tripeptide **36c′** (intramolecular hydrogen bond) and **39c′** (without intramolecular hydrogen bond).

Table 2
Chemical shifts of the amidic NH protons in tripeptides **35**/**35′**, **36**/**36′** and **38**/**38′**–**42**/**42′**

Entry	Compound	δ (ppm)	
		6′-NH	2-NH
1	**35a**/**35a′**	5.48/5.60	7.88/8.04
2	**35c**/**35c′**	5.91/5.99	7.84/8.08
3	**36c**/**36c′**	5.21/5.52	8.48/8.39
4	**38c**/**38c′**	6.51/6.59	4.92/5.05
5	**39c**/**39c′**	6.62/6.55	5.11/4.94
6	**40b**/**40b′**	5.30/5.26	7.62/7.53
7	**41d**/**41d′**	5.53/5.53	9.02/9.27
8	**42d**/**42d′**	6.80/6.97	5.09/5.12

2.5. Biological activity

Compounds **8a**,**c**, **9a**,**b**,**d**, **10a**, **11b**, **15a**, **16b**, **17b**,**d**, **20a**, **21b**,**d**, **35a**, **35a′**, **40b** and **40b′** were also tested for inhibitory activities on two bacterial peptidoglycan biosynthesis enzymes: MurD ligase (MurD) and D-alanine:D-alanine ligase (DdlB).[18] The Malachite green assay,[19] which detects the orthophosphate generated during

enzymatic reactions was used. The results are presented as residual activities (RAs) of the enzyme in the presence of 250 μM of each compound. Compounds **9a**, **9d**, **13c**, **18c**, **21b**, **35a** and **35a′** showed a weak inhibition of MurD (entries 3, 5, 8, 14, 17, 27 and 28), whereas compounds **21b** and **35a′** showed a weak inhibition of DdlB (entries 17 and 28). The other compounds did not inhibit these two enzymes (Table 3).

Table 3
Inhibitory activity of compounds **8a**,**c**, **9a**,**b**,**d**, **10a**, **11b**, **15a**, **16b**, **17b**,**d**, **20a**, **21b**,**d**, **35a**, **35a′**, **40b** and **40b′** against MurD ligase and D-Ala:D-Ala ligase

Entry	Compound	% RA	
		MurD	Ddl
1	**8a**	77[a]	94
2	**8c**	93	90
3	**9a**	63[a]	98
4	**9b**	81[a]	96
5	**9d**	47	77
6	**10a**	81	nd[b]
7	**11b**	78[a]	140
8	**13c**	49[a]	86[a]
9	**14d**	101	100
10	**15a**	98[a]	88[a]
11	**16b**	97	96[a]
12	**17b**	102[a]	91
13	**17d**	109	100
14	**18c**	51[a]	96
15	**19d**	69[a]	99
16	**20a**	75	87
17	**21b**	46	66
18	**21d**	82	102
19	**22c**	95	98
20	**23d**	65	98
21	**25**	99	102[a]
22	**26**	96	99
23	**28**	107	93
24	**30**	99	100
25	**31**	98	93
26	**33**	101	98
27	**35a**	60[a]	92
28	**35a′**	63[a]	70[a]
29	**40b**	nd[b]	96
30	**40b′**	77[a]	nd[b]

[a] Slight precipitation occurred.
[b] Not determined.

3. Conclusion

A series of tetrahydropyrazolo[1,2-*a*]pyrazole-based peptide mimetics has been synthesized in five steps from methyl *N*-benzyloxycarbonyl-α,β-dehydro-β-phenylalaninate (**2a**) and methyl *N*-benzyloxycarbonyl-α,β-dehydroleucinate (**2b**). The synthesis starts with a two step preparation of 5-substituted 1-benzylidene-4-benzyloxycarbonylamino-3-oxopyrazolidin-1-ium-2-ides **5a**–**d**, followed by 1,3-dipolar cycloaddition to *tert*-butyl acrylate (**6a**) and *tert*-butyl methacrylate (**6b**) to afford the corresponding racemic cycloadducts **8**–**14** as heterocyclic dipeptides that are selectively deprotectable at the C- and the N-terminal. Using conventional peptide chemistry protocols, tetrapeptide **28** and hexapeptide **33** with 6-amino-7-oxotetrahydropyrazolo[1,2-*a*]pyrazole-1-carboxylic acid (**1**) as the central dipeptide unit were prepared. Besides, coupling of racemic carboxy-dipeptides **15**, **16**, **18** and **19** with methyl (*S*)-alaninate (**34**) and coupling of amino-dipeptides **20**, **22** and **23** with *N*-Boc-(*S*)-alanine (**37**) gave the corresponding tripeptides as 1:1 mixtures of diastereomers, which were separated by MPLC to furnish diastereomerically pure non-racemic title compounds **35**/**35′**, **36**/**36′** and **38**/**38′**–**42**/**42′**. The synthesized compounds were also evaluated for inhibition of MurD ligase and D-alanine:D-alanine ligase, however, only a few compounds showed a weak inhibition of these two enzymes. In conclusion, this synthetic method enables preparation of structurally and stereochemically diverse heterocyclic dipeptides that can be easily incorporated into peptides. Somewhat low regio- and stereo-selectivity of cycloadditions is not necessarily disadvantageous, since higher number of separable isomeric cycloadducts increases the stereochemical diversity of heterocyclic dipeptides and compounds derived thereof.

4. Experimental

4.1. General

Melting points were determined on a Stanford Research Systems MPA100 OptiMelt automated melting point system. The NMR spectra were obtained on a Bruker Avance DPX 300 at 300 MHz for ^{1}H and 75.5 MHz for ^{13}C and on a Bruker Avance III UltraShield 500 plus at 500 MHz for ^{1}H and 126 MHz for ^{13}C, using acetone-d_6, acetonitrile-d_3, $CDCl_3$, and DMSO-d_6 with Me_4Si as the internal standard, as solvents. Mass spectra were recorded on a Q-Tof Premier spectrometer and on a Agilent 6224 Accurate Mass TOF LC/MS spectrometer, IR spectra on a Perkin–Elmer Spectrum BX FTIR spectrophotometer. Microanalyses were performed on a Perkin–Elmer CHN Analyzer 2400 II. Flash column chromatography (FC) was performed on silica gel (Fluka, Silica gel 60, particle size 35–70 μm). Medium pressure liquid chromatography (MPLC) was performed on a Büchi Flash Chromatography System (Büchi Fraction Collector C-660, Büchi Pump Module C-605, Büchi Control Unit C-620) on silica gel (LiChroprep® Si 60, 15–25 μm), column dimensions: 36×460 mm, backpressure: 10 Bar, detection: UV (254 nm).

tert-Butyl acrylate (**6a**), *tert*-butyl methacrylate (**6b**), 10% Pd–C and EEDQ are commercially available (Sigma Aldrich). Methyl 2-(benzyloxycarbonylamino)-3-phenylacrylate (**2a**), methyl 2-(benzyloxycarbonylamino)-3-isopropylacrylate (**2b**),[14] pyrazolidinone **3a** and azomethine imines **5a**,**b**[15] were prepared following the literature procedures.

4.2. (4*R**,5*R**)-4-Benzyloxycarbonylamino-5-isopropylpyrazolidin-3-one (3b)

A mixture of **2b** (22 g, 80 mmol), ethanol (120 mL) and hydrazine hydrate (11.66 mL, 240 mmol) was stirred at rt for 3 h. The precipitate was collected by filtration and washed with ethanol (15 mL) to give **3b**. Yield: 18.16 g (83%) of pale yellow solid; mp 159–163 °C; [Found: C, 60.54; H, 6.84; N, 15.03. $C_{14}H_{19}N_3O_3$ requires: C, 60.63; H, 6.91; N, 15.15%]; ν_{max} (KBr) 3450, 3320, 3213, 2951, 1721 (C=O), 1697 (C=O), 1661, 1635, 1542, 1472, 1457, 1418, 1356, 1292, 1244, 1167, 1061, 978, 775, 756, 730, 696, 645 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 0.89 and 0.98 (6H, 2d, 1:1, J=6.7 Hz, Me_2CH), 1.77 (1H, septet, J=6.4 Hz, C*H*Me$_2$), 2.92 (1H, td, J=8.7, 10.7 Hz, 5-H), 4.02 (1H, dd, J=9.4, 10.7 Hz, 4-H), 4.89 (1H, d, J=11.0 Hz, 1-H), 5.04 and 5.07 (2H, 2d, 1:1, J=12.6 Hz, CH_2Ph), 7.30–7.40 (5H, m, Ph), 7.60 (1H, d, J=9.4 Hz, NH), 9.22 (1H, s, 2-H); δ_C (126 MHz, DMSO-d_6) 18.8, 19.6, 30.10, 56.1, 65.4, 68.0, 127.7, 127.8, 128.4, 137.1, 156.1, 173.7; HRMS (ESI): MH$^+$, found 278.1509. $C_{14}H_{20}N_3O_3$ requires 278.1505.

4.3. General procedure for the preparation of azomethine imines 5c,d

A mixture of **3b** (2.77 g, 10 mmol), aromatic aldehyde **4** (12 mmol) and methanol (40 mL) was stirred at room temperature for 5 min. Then, trifluoroacetic acid (20 drops) was added and the mixture was stirred at rt for 7 h. The precipitate was collected by filtration and washed with methanol (10 mL) to give **5**.

The following compounds were prepared in this manner.

4.3.1. (3R,4R*,Z)-2-Benzylidene-4-(benzyloxycarbonylami-no)-3-isopropyl-5-oxopyrazolidin-2-ium-1-ide (**5c**).* Prepared from **3b**

(2.77 g, 10 mmol) and benzaldehyde (**4a**) (1.22 mL, 12 mmol). Yield: 3.50 g (96%) of white solid; mp 209–213 °C; [Found: C, 69.13; H, 6.26; N, 11.51. $C_{21}H_{23}N_3O_3$ requires C, 69.02; H, 6.34; N, 11.50%]; ν_{max} (KBr) 3420, 3187, 2959, 1713 (C=O), 1656 (C=O), 1589, 1569, 1451, 1383, 1327, 1276, 1162, 1097, 1042, 747, 676 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 0.76 and 1.04 (6H, 2d, 1:1, J=6.8 Hz, Me_2CH), 2.52–2.57 (1H, m, C*H*Me$_2$), 4.09 (1H, dd, J=4.4, 8.5 Hz, 4-H), 4.48 (1H, dd, J=4.4, 1.1 Hz, 3-H), 5.05 (2H, s, CH_2Ph), 7.30–7.37 (4H, m, 4H of Ph), 7.38 (1H, br s, 1′-H), 7.60 (3H, dd, J=1.9, 5.2 Hz, 3H of Ph), 7.80 (1H, s, NH), 7.90 (1H, d, J=8.5 Hz, 1H of Ph), 8.35 (2H, dd, J=2.8, 6.6 Hz, 2H of Ph); δ_C (75.5 MHz, DMSO-d_6) 14.1, 18.0, 30.6, 51.0, 65.6, 78.1, 127.5, 127.6, 128.1, 128.3, 129.4, 131.3, 131.4, 133.4, 136.5, 155.6, 180.3; HRMS (ESI): MH^+, found 366.1806. $C_{21}H_{24}N_3O_3$ requires 366.1812.

4.3.2. (3R,4R*,Z)-4-(Benzyloxycarbonylamino)-2-(2,6-dichloro-benzylidene)-3-isopropyl-5-oxopyrazolidin-2-ium-1-ide (**5d**).* Prepared from **3b** (2.77 g, 10 mmol) and 2,6-dichlorobenzaldehyde (**4b**) (2.10 g, 12 mmol). Yield: 4.30 g (99%) of white solid; mp 180–185 °C; [Found: C, 57.89; H, 4.72; N, 9.61. $C_{21}H_{21}Cl_2N_3O_3$ requires C, 58.07; H, 4.87; N, 9.68%]; ν_{max} (KBr) 3433, 3193, 3027, 2969, 2934, 1720 (C=O), 1670 (C=O), 1580, 1558, 1463, 1432, 1375, 1330, 1315, 1274, 1258, 1172, 1114, 1092, 1040, 1018, 808, 795, 741, 708, 698, 673 cm^{-1}; δ_H (500 MHz, DMSO-d_6) 0.92 and 1.06 (6H, 2d, 1:1, J=6.8 Hz, Me_2CH), 2.63–2.70 (1H, m, C*H*Me$_2$), 4.17 (1H, dd, J=4.8, 8.4 Hz, 4-H), 4.85 (1H, dd, J=4.8, 1.0 Hz, 3-H), 5.06 (2H, s, CH_2Ph), 7.27–7.33 (1H, m, p-C_6H_3), 7.33–7.37 (4H, m, 4H of Ar), 7.46–7.50 (3H, m, 3H of Ar), 7.88 (1H, br s, 1′-H), 7.95 (1H, d, J=8.4 Hz, NH); δ_C (75.5 MHz, DMSO-d_6) 14.1, 18.1, 30.3, 51.4, 65.6, 77.1, 127.4, 127.5, 127.8, 128.0, 128.3, 128.6, 128.9, 132.4, 133.5, 155.71, 180.0; HRMS (ESI): MH^+, found 434.1031. $C_{21}H_{22}Cl_2N_3O_3$ requires 434.1033.

4.4. General procedure for the synthesis of *tert*-butyl 6-(benzyloxycarbonylamino)-7-oxotetrahydropyrazolo[1,2-*a*]-pyrazole-1(or 2) carboxylates (racemic fully protected dipeptides)

Under argon, a mixture of dipole **5a**–**d** (5 mmol), *tert*-butyl acrylate (**6a**)(0.87 mL, 6 mmol) or *tert*-butyl methacrylate (**6b**)(0.98 mL, 6 mmol) and anisole (25 mL) was heated under reflux for 4–7 h. (Reactions of 5-phenyl substituted dipoles **5a**,**b** produced the insoluble hydantoin by-products **7a**,**b**, which were isolated by filtration, while the filtrate was subjected to further workup.) Volatile components were evaporated in vacuo and the residue was purified by FC (EtOAc). Fractions containing the products were combined and evaporated in vacuo to give a mixture of cycloadducts **8**–**12**, which were separated by MPLC (EtOAc/hexanes). Fractions containing the products were combined and evaporated in vacuo to give isomerically pure compounds **8**–**12**.

The following compounds were prepared in this manner.

*4.4.1. Synthesis of hydantoin derivative **7a** and cycloadducts **8a**, **9a** and **10a**.* Prepared from **5a** (0.799 g, 2 mmol), *tert*-butyl acrylate (**6a**, 0.348 mL, 2.4 mmol) and anisole (12 mL), 4 h, MPLC (EtOAc/hexanes, 1:3).

*4.4.1.1. (Z)-5-Benzylidene-3-((E)-benzylideneamino)imidazolidine-2,4-dione (**7a**).* Yield: 0.160 g (28%) of white solid. Physical, spectral and analytical data for compound **7a** were in agreement with the literature data.[15]

4.4.1.2. tert-Butyl (1R,3R*,5R*,6R*)-6-(benzyloxycarbonylamino)-7-oxo-3,5-diphenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**8a**).* Yield: 0.305 g (29%) of white solid; mp 130–135 °C; [Found: C, 70.54; H, 6.36; N, 7.72. $C_{31}H_{33}N_3O_5$ requires C, 70.57; H, 6.30; N, 7.96%]; ν_{max} (KBr) 3391, 3064, 3035, 2976, 2930, 1736, 1716, 1542, 1497, 1457, 1369, 1249, 1229, 1153, 1065, 1057, 754, 742, 697 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.55 (9H, s, *t*-Bu), 2.50 (1H, ddd, J=0.8, 5.5, 13.5 Hz, 2-Ha), 2.74 (1H, ddd, J=9.3, 11.5, 13.5 Hz, 2-Hb), 3.99 (1H, dd, J=5.5, 11.5 Hz, 3-H), 4.05 (1H, d, J=12.0 Hz, 5-H), 4.41 (1H, dd, J=0.8, 9.3 Hz, 1-H), 4.97 (2H, s, CH_2Ph), 5.06 (1H, m, 6-H), 5.30 (1H, br d, J=8.4 Hz, NH), 7.95–6.68 (15H, m, 3×Ph); δ_H (500 MHz, MeCN-d_3) 1.53 (9H, s, *t*-Bu), 2.56 (1H, ddd, J=1.2, 5.6, 13.2 Hz, 2-Ha), 2.71 (1H, ddd, J=9.4, 11.5, 13.2 Hz, 2-Hb), 3.98 (1H, dd, J=5.6, 11.5 Hz, 3-H), 4.09 (1H, d, J=13.0 Hz, 5-H), 4.29 (1H, dd, J=1.2, 9.4 Hz, 1-H), 4.82 (1H, ddd, J=1.1, 9.8, 13.0 Hz, 6-H), 4.96 (2H, s, CH_2Ph), 6.14 (1H, br d, J=9.8 Hz, NH), 7.02–7.05 (6H, m, 6H of Ph), 7.18–7.3 (9H, m, 9H of Ph); δ_C (126 MHz, $CDCl_3$) 28.1, 43.0, 55.2, 63.0, 67.1, 68.8, 79.0, 83.3, 127.7, 128.0, 128.0, 128.1, 128.1, 128.1, 128.1, 128.3, 128.5, 135.0, 136.2, 136.3, 155.8, 162.9, 168.9; HRMS (ESI): MH^+, found 528.2493. $C_{31}H_{34}N_3O_5$ requires 528.2498.

4.4.1.3. tert-Butyl (1S,3S*,5R*,6R*)-6-(benzyloxycarbonylamino)-7-oxo-3,5-diphenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**9a**).* Yield: 53 mg (5%) of colourless oil; ν_{max} (liquid film) 3326, 3059, 3033, 2977, 2925, 1731, 1532, 1496, 1456, 1390, 1369, 1287, 1250, 1155, 1063, 1026, 760, 738, 697 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.51 (9H, s, *t*-Bu), 2.31 (1H, br sextet, J=7.5 Hz, 2-Ha), 2.95 (1H, br td, J=9.2, 13.0 Hz, 2-Hb), 3.65 (1H, t, J=8.1 Hz, 3-H), 4.43 (1H, d, J=7.4 Hz, 5-H), 4.47 (1H, dd, J=6.2, 10.0 Hz, 1-H), 5.08 (2H, s, CH_2Ph), 5.04–5.11 (1H, m, 6-H, overlapped by the signal for CH_2Ph), 5.47 (1H, br d, J=8.5 Hz, NH), 6.74–7.55 (15H, m, 3×Ph); δ_H (500 MHz, Me_2CO-d_6) 1.51 (9H, s, *t*-Bu), 2.21 (1H, ddd, J=6.1, 8.1, 13.1 Hz, 2-Ha), 3.02 (1H, ddd, J=8.0, 10.0, 13.1 Hz, 2-Hb), 4.09 (1H, t, J=8.0 Hz, 3-H), 4.52 (1H, dd, J=6.1, 10.0 Hz, 1-H), 4.74 (1H, d, J=11.3 Hz, 5-H), 5.03 and 5.07 (2H, 2d, 1:1, J=12.5 Hz, CH_2Ph), 5.23 (1H, dd, J=9.6, 11.3 Hz, 6-H), 6.95–7.07 (8H, m, NH and 7H of Ph), 7.17–7.35 (8H, m, 8H of Ph); δ_C (126 MHz, $CDCl_3$) 28.1, 42.8, 54.3, 59.2, 62.2, 66.6, 67.4, 83.0, 127.8, 127.8, 128.3, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 133.2, 136.1, 137.9, 156.1, 167.3, 168.5; HRMS (ESI): MH^+ found 528.2514. $C_{31}H_{34}N_3O_5$ requires 528.2498.

4.4.1.4. tert-Butyl (2R,3R*,5R*,6R*)-6-(benzyloxycarbonylamino)-7-oxo-3,5-diphenylhexahydropyrazolo[1,2-a]pyrazole-2-carboxylate (**10a**).* Yield: 116 mg (11%) of colourless oil; ν_{max} (liquid film) 3296, 3064, 2977, 2930, 1731, 1693, 1531, 1497, 1456, 1393, 1368, 1308, 1252, 1217, 1153, 1059, 1029, 1003, 844, 764, 737, 698, 632 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.35 (9H, s, *t*-Bu), 3.38 (1H, br dt, J=7.0, 9.2 Hz, 2-H), 3.72 (1H, br t, J=10.7 Hz, 1-Ha), 3.99 (1H, br d, J=8.2 Hz, 3-H), 4.15 (1H, br d, J=11.9 Hz, 5-H), 4.19 (1H, dd, J=7.0, 11.6 Hz, 1-Hb), 4.69 (1H, br t, J=9.9 Hz, 6-H), 5.05 (2H, s, CH_2Ph), 5.25 (1H, br d, J=8.0 Hz, NH), 7.07–7.30 (15H, m, 3×Ph); δ_H (500 MHz, Me_2CO-d_6) 1.32 (9H, s, *t*-Bu), 3.52 (1H, dt, J=7.2, 9.5 Hz, 2-H), 3.71 (1H, ddd, J=1.4, 9.5, 11.2 Hz, 1-Ha), 4.00 (1H, d, J=9.5 Hz, 3-H), 4.08 (1H, dd, J=7.2, 11.2 Hz, 1-Hb), 4.24 (1H, d, J=10.5 Hz, 5-H), 4.62 (1H, d, J=10.5 Hz, 6-H), 5.05 and 5.06 (2H, 2d, 1:1, J=12.6 Hz, CH_2Ph), 7.06–7.14 (6H, m, 6H of Ph), 7.25–7.36 (9H, m, 9H of Ph), NH exchanged; δ_C (126 MHz, $CDCl_3$) 28.1, 43.3, 54.7, 63.6, 67.3, 73.8, 74.7, 82.4, 127.7, 128.0, 128.2, 128.2, 128.2, 128.2, 128.3, 128.3, 128.7, 136.2, 136.3, 136.9, 155.9, 163.1, 169.7; HRMS (ESI): MH^+, found 528.2508. $C_{31}H_{34}N_3O_5$ requires 528.2498.

*4.4.2. Synthesis of hydantoin derivative **7b** and cycloadducts **9b** and **11b**.* Prepared from **5b** (0.936 g, 2 mmol), *tert*-butyl acrylate (**6a**)(0.348 mL, 2.4 mmol), and anisole (12 mL), 4 h, MPLC (EtOAc/hexanes, 1:2).

*4.4.2.1. (Z)-5-Benzylidene-3-((E)-(2,6-dichlorobenzylidene)amino)imidazolidine-2,4-dione (**7b**).* Yield: 0.144 g (20%) of white solid. Physical, spectral and analytical data for compound **7b** were in agreement with the literature data.[15]

4.4.2.2. tert-Butyl (1S,3S*,5R*,6R*)-6-(benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-7-oxo-5-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**9b**).* Yield: 166 mg (14%) of colourless oil; ν_{max}

(liquid film) 3255, 3149, 3064, 2980, 1747, 1726, 1718, 1700, 1686, 1563, 1445, 1408, 1368, 1337, 1214, 1152, 1145, 1131, 1091, 1077, 1048, 1042, 1029, 782, 769, 760, 755, 729, 698 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.50 (9H, s, *t*-Bu), 2.70 (1H, ddd, *J*=5.8, 9.8, 13.1 Hz, 2-Ha), 2.75 (1H, ddd, *J*=8.9, 10.1, 13.1 Hz, 2-Hb), 4.39 (1H, t, *J*=9.2 Hz, 3-H), 4.72 (1H, dd, *J*=5.8, 10.1 Hz, 1-H), 4.87 (1H, d, *J*=10.7 Hz, 5-H), 5.11 (2H, s, CH_2Ph), 5.15 (1H, d, *J*=9.0 Hz, NH), 5.40 (1H, br t, *J*=10.7 Hz, 6-H), 6.80–7.72 (13H, m, 2× Ph and C_6H_3); δ_H (500 MHz, Me_2CO-d_6) 1.50 (9H, s, *t*-Bu), 2.67 (1H, ddd, *J*=5.5, 9.5, 12.8 Hz, 2-Ha), 2.93 (1H, ddd, *J*=8.6, 10.7, 12.8 Hz, 2-Hb), 4.64 (1H, t, *J*=9.1 Hz, 3-H), 4.69 (1H, dd, *J*=5.5, 10.6 Hz, 1-H), 4.96 (1H, d, *J*=12.3 Hz, 5-H), 5.12 (2H, s, CH_2Ph), 5.37 (1H, dd, *J*=9.6, 12.3 Hz, 6-H), 6.87 (1H, d, *J*=7.8 Hz, 1H of Ph), 6.92–7.03 (5H, m, NH and 4H of Ph), 7.18 (1H, d, *J*=7.5 Hz, 1H of Ph), 7.24–7.40 (7H, m, 7H of Ph), 7.64–7.67 (1H, m, 1H of Ph), 7.73–7.77 (1H, m, 1H of Ph); δ_C (126 MHz, $CDCl_3$) 28.1, 36.4, 52.5, 55.4, 59.3, 64.3, 67.6, 82.6, 128.1, 128.30, 128.34, 128.4, 128.6, 128.8, 128.9, 130.8, 131.0, 132.1, 132.6, 136.0, 167.9, 168.7, 175.0; HRMS (ESI): MH^+, found 596.1733. $C_{31}H_{32}Cl_2N_3O_5$: requires 596.1719.

4.4.2.3. tert-Butyl (2S,3S*,5R*,6R*)-6-(benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-7-oxo-5-phenylhexahydropyrazolo[1,2-a]pyrazole-2-carboxylate (**11b**).* Yield: 418 mg (36%) of colourless crystals; mp 182–186 °C; [Found: C, 62.44; H, 5.20; N, 7.15. $C_{31}H_{31}Cl_2N_3O_5$ requires C, 62.42; H, 5.24; N, 7.04%]; ν_{max} (liquid film) 3331, 3070, 2977, 2930, 1730, 1705, 1562, 1525, 1476, 1456, 1443, 1370, 1319, 1295, 1253, 1217, 1184, 1157, 1110, 1049, 1023, 977, 787, 761, 697, 625 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.42 (9H, s, *t*-Bu), 3.60 (1H, m, 2-H), 3.87 (1H, br t, *J*=10.6 Hz, 1-Ha), 4.11 (1H, br dd, *J*=4.1, 11.9 Hz, 1-Hb), 4.59 (1H, d, *J*=11.1 Hz, 5-H), 4.98 (1H, d, *J*=6.8 Hz, 3-H), 5.07 (2H, s, CH_2Ph), 5.21–5.32 (2H, m, NH and 6-H), 6.86–7.29 (13H, m, 2× Ph and C_6H_3); δ_C (126 MHz, $CDCl_3$) 27.9, 45.6, 52.3, 54.7, 62.0, 65.8, 67.5, 82.7, 128.17, 128.28, 128.34, 128.4, 128.5, 128.6, 129.31, 129.33, 130.1, 132.1, 132.9, 135.0, 156.2, 170.9, 172.1; HRMS (ESI): MH^+, found 596.1742. $C_{31}H_{32}Cl_2N_3O_5$: requires 596.1719.

*4.4.3. Synthesis of cycloadducts **8c** and **10c**.* Prepared from **5c** (0.730 g, 2 mmol), *tert*-butyl acrylate (**6a**)(0.348 mL, 2.4 mmol), and anisole (12 mL), 7 h, MPLC (EtOAc/hexanes, 1:3).

4.4.3.1. tert-Butyl (1R,3S*,5R*,6R*)-6-(benzyloxycarbonylamino)-5-isopropyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**8c**).* Yield: 0.326 g (33%) of white solid; mp 153–156 °C; [Found: C, 68.08; H, 7.10; N, 8.51. $C_{28}H_{35}N_3O_5$ requires C, 68.13; H, 7.15; N, 8.51%]; ν_{max} (KBr) 3370, 2968, 1732, 1715, 1698, 1526, 1496, 1458, 1413, 1381, 1371, 1305, 1247, 1235, 1216, 1152, 1060, 1022, 752, 701 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.75 and 0.86 (6H, 2d, 1:1, *J*=6.8 Hz, Me_2CH), 1.37 (1H, septet, *J*=6.9 Hz, C*H*Me_2), 1.52 (9H, s, *t*-Bu), 2.49 (1H, ddd, *J*=0.7, 5.6, 13.2 Hz, 2-Ha), 2.65 (1H, ddd, *J*=9.3, 11.5, 13.2 Hz, 2-Hb), 3.11 (1H, dd, *J*=2.7, 11.8 Hz, 5-H), 3.88 (1H, dd, *J*=5.6, 11.5 Hz, 3-H), 4.31 (1H, dd, *J*=0.7, 9.3 Hz, 1-H), 4.93 (1H, dd, *J*=9.5, 11.8 Hz, 6-H), 5.08 and 5.14 (2H, 2d, 1:1, *J*=12.3 Hz, CH_2Ph), 5.22 (1H, br d, *J*=9.5 Hz, NH), 7.04–7.33 (10H, m, 2× Ph); δ_C (126 MHz, $CDCl_3$) 16.3, 18.4, 27.4, 28.1, 44.0, 55.3, 56.1, 67.3, 69.0, 80.2, 83.2, 127.5, 128.2, 128.3, 128.6, 128.6, 128.9, 136.3, 138.2, 156.0, 164.3, 169.0; HRMS (ESI): MH^+, found 494.2641. $C_{28}H_{36}N_3O_5$ requires 494.2649.

4.4.3.2. tert-Butyl (2R,3R*,5R*,6R*)-6-(benzyloxycarbonylamino)-5-isopropyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-2-carboxylate (**10c**).* Yield: 69 mg (7%) of colourless oil; ν_{max} (liquid film) 3284, 3062, 3035, 2966, 2875, 1732, 1694, 1682, 1538, 1456, 1392, 1369, 1254, 1153, 1112, 1082, 1056, 737, 700 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.76 and 0.81 (6H, 2d, 1:1, *J*=6.6 Hz, Me_2CH), 1.39 (9H, s, *t*-Bu), 1.55 (1H, m, C*H*Me_2), 3.04 (1H, dd, *J*=3.6, 9.4 Hz, 5-H), 3.27 (1H, br dt, *J*=6.2, 8.9, 2-H), 3.56 (1H, br t, *J*=10.5 Hz, 1-Ha), 3.90 (1H, d, *J*=8.0 Hz, 3-H), 4.14 (1H, dd, *J*=6.2, 11.7 Hz, 1-Hb), 4.60 (1H, t, *J*=8.9 Hz, 6-H), 5.11 and 5.15 (2H, 2d, 1:1, *J*=12.3 Hz, CH_2Ph), 5.27 (1H, d, *J*=7.8 Hz, NH), 7.30–7.39 (10H, m, 2× Ph); δ_C (126 MHz, $CDCl_3$) 17.5, 18.1, 28.0, 29.8, 43.4, 54.8, 56.4, 67.3, 74.1, 75.2, 82.3, 128.1, 128.3, 128.3, 128.6, 128.7, 128.7, 136.2, 138.0, 155.9, 166.3, 170.3; HRMS (ESI): MH^-, found 492.2526. $C_{28}H_{34}N_3O_5$ requires 492.2504.

*4.4.4. Synthesis of cycloadducts **9d**–**12d**.* Prepared from **5d** (0.866 g, 2 mmol), *tert*-butyl acrylate (**6a**)(0.348 mL, 2.4 mmol), and anisole (12 mL), 5 h, MPLC (EtOAc/hexanes, 1:3).

4.4.4.1. tert-Butyl (1S,3S*,5R*,6R*)-6-(benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-5-isopropyl-7-oxohexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**9d**).* Yield: 101 mg (9%) of pale yellow crystals; mp 150–155 °C; [Found: C, 59.92; H, 6.06; N, 7.43. $C_{28}H_{33}Cl_2N_3O_5$ requires C, 59.79; H, 5.91; N, 7.47%]; ν_{max} (KBr) 3254, 3057, 2977, 2964, 1879, 1751, 1725, 1682, 1578, 1540, 1455, 1440, 1392, 1369, 1336, 1282, 1235, 1144, 1007, 1084, 1051, 1038, 986, 997, 842, 794, 784, 759, 702 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.83 and 0.97 (6H, 2d, 1:1, *J*=6.8 Hz, Me_2CH), 1.50 (9H, s, *t*-Bu), 1.80–1.90 (1H, m, C*H*Me_2), 2.59 (1H, dd, *J*=3.9, 9.0 Hz, 5-H), 2.97–3.06 (2H, m, 2-CH_2), 4.84 (1H, br t, *J*=9.0 Hz, 6-H), 4.85 (1H, br dd, *J*=3.1, 9.5 Hz, 3-H), 5.05 and 5.10 (2H, 2d, 1:1, *J*=12.2 Hz, CH_2Ph), 5.11 (1H, d, *J*=9.0 Hz, NH), 5.55 (1H, dd, *J*=5.8, 8.7 Hz, 1-H), 7.19 (1H, t, *J*=8.0 Hz, *p*-C_6H_3), 7.29–7.41 (7H, m, Ph and *m*-C_6H_3); δ_C (126 MHz, $CDCl_3$) 17.9, 18.1, 28.1, 30.5, 35.1, 53.6, 57.4, 59.5, 67.3, 69.7, 82.9, 128.4, 128.7, 129.8, 130.6, 133.6, 133.9, 136.1, 138.0, 155.9, 162.1, 168.7; HRMS (ESI): MNa^+, found 584.1677. $C_{28}H_{33}Cl_2N_3NaO_5$ requires 584.1689.

4.4.4.2. tert-Butyl (2R,3R*,5R*,6R*)-6-(benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-5-isopropyl-7-oxohexahydropyrazolo[1,2-a]pyrazole-2-carboxylate (**10d**).* Yield: 101 mg (9%) of colourless oil; ν_{max} (liquid film) 3414, 2962, 1735, 1725, 1682, 1531, 1539, 1250, 1452, 1438, 1384, 1369, 1328, 1261, 1156, 1144, 1087, 1049, 783, 770, 698 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.71 and 0.76 (6H, 2d, 1:1, *J*=6.7 Hz, Me_2CH), 1.40 (9H, s, *t*-Bu), 1.54–1.63 (1H, m, C*H*Me_2), 3.04 (1H, dd, *J*=4.5, 7.6 Hz, 5-H), 3.65 (1H, ddd, *J*=1.5, 9.7, 11.6 Hz, 1-Ha), 3.89 (1H, ddd, *J*=3.9, 7.9, 9.7 Hz, 2-H), 4.37 (1H, br dd, *J*=3.9, 11.6 Hz, 1-Hb), 4.47 (1H, br t, *J*=7.6 Hz, 6-H), 4.95 (1H, br d, *J*=7.9 Hz, 3-H), 5.14 (2H, 2d, 1:1, *J*=12.4 Hz, CH_2Ph), 5.44 (1H, d, *J*=7.6 Hz, NH), 7.27–7.42 (8H, m, Ph and C_6H_3); δ_C (126 MHz, $CDCl_3$) 17.7, 18.2, 30.9, 43.7, 50.0, 57.2, 67.2, 68.7, 74.6, 77.4, 82.5, 128.2, 128.3, 128.6, 128.7, 130.0, 130.8, 136.2, 136.8, 155.9, 167.4, 170.9; HRMS (ESI): MH^+, found 562.1868. $C_{28}H_{34}Cl_2N_3O_5$ requires 562.1870.

4.4.4.3. tert-Butyl (2S,3S*,5R*,6R*)-6-(benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-5-isopropyl-7-oxohexahydropyrazolo[1,2-a]pyrazole-2-carboxylate (**11d**).* Yield: 292 mg (26%) of colourless crystals; mp 155–159 °C; [Found: C, 59.52; H, 5.87; N, 7.36. $C_{28}H_{33}Cl_2N_3O_5$ requires C, 59.79; H, 5.91; N, 7.47%]; ν_{max} (KBr) 3329, 2976, 2933, 1728, 1561, 1536, 1454, 1437, 1392, 1368, 1298, 1251, 1230, 1149, 1121, 1056, 853, 819, 778, 758, 734, 696 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.68 and 0.93 (6H, 2d, 1:1, *J*=6.7 Hz, Me_2CH), 1.45 (9H, s, *t*-Bu), 1.81 (1H, br sextet, *J*=6.7 Hz, C*H*Me_2), 2.78 (1H, dd, *J*=6.4, 10.1 Hz, 5-H), 3.69–3.78 (2H, m, 1-Ha and 2-H), 4.11–4.19 (1H, m, 1-Hb), 4.75 (1H, t, *J*=9.9 Hz, 6-H), 5.07 (1H, d, *J*=8.6 Hz, NH), 5.07 and 5.15 (2H, 2d, 1:1, *J*=12.2 Hz, CH_2Ph), 5.49 (1H, d, *J*=5.8 Hz, 3-H), 7.21–7.41 (8H, m, Ph and C_6H_3); δ_H (500 MHz, Me_2CO-d_6) 0.47 and 0.94 (6H, 2d, 1:1, *J*=6.7 Hz, Me_2CH), 1.42 (9H, s, *t*-Bu), 1.84–1.95 (1H, m, C*H*Me_2), 3.31 (1H, ddd, *J*=1.7, 10.1, 11.4 Hz, 5-H), 3.53 (1H, ddd, *J*=3.2, 6.6, 9.4 Hz, 2-H), 3.77 (1H, dd, *J*=9.4, 11.6, 1-Ha), 4.09 (1H, dd, *J*=3.2, 11.6 Hz, 1-Hb), 4.57 (1H, d, *J*=11.4 Hz, 6-H), 5.07 and 5.15 (2H, 2d, 1:1, *J*=12.4 Hz, CH_2Ph), 5.14 (1H, d, *J*=6.8 Hz, 3-H), 6.82 (1H, d, *J*=9.3 Hz, NH), 7.28–7.44 (6H, m, Ph and *p*-C_6H_3). 7.47–7.57 (2H, m, *m*-C_6H_3); δ_C (126 MHz, $CDCl_3$) 18.5, 19.2, 28.0, 29.9, 43.4, 51.2, 56.2, 62.8, 67.4, 69.7, 82.8, 128.4, 128.7, 129.7, 130.7, 133.3, 134.7, 136.2,

137.5, 156.0, 167.0, 170.5; HRMS (ESI): MH^+, found 562.1862. $C_{28}H_{34}Cl_2N_3O_5$ requires 562.1780.

4.4.4.4. tert-Butyl (1R,3S*,5R*,6R*)-6-(benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-5-isopropyl-7-oxohexahydropyrazolo[1,2-a] pyrazole-1-carboxylate (**12d**).* Yield: 135 mg (12%) of colourless oil; ν_{max} (liquid film) 3329, 3066, 2976, 2934, 2873, 1728, 1581, 1561, 1536, 1455, 1438, 1393, 1369, 1298, 1252, 1230, 1149, 1121, 1090, 1057, 1042, 778, 758, 735, 697 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.53 and 0.90 (6H, 2d, 1:1, *J*=6.7 Hz, *Me*$_2$CH), 1.68 (9H, s, *t*-Bu), 1.73 (1H, m, C*H*Me$_2$), 2.81 (2H, br t, *J*=8.5 Hz, 2-CH_2), 3.26 (1H, t, *J*=10.1 Hz, 5-H), 4.64 (1H, br t, *J*=9.1 Hz, 3-H), 4.66 (1H, br t, *J*=10.1 Hz, 6-H), 4.71 (1H, br t, *J*=7.9 Hz, 1-H), 5.06 (1H, d, *J*=9.8 Hz, NH), 5.12 and 5.18 (2H, 2d, 1:1, *J*=12.2 Hz, C*H*$_2$Ph), 7.17 (1H, t, *J*=8.0 Hz, *p*-C_6H_3), 7.28–7.43 (7H, m, Ph and *m*-C_6H_3); δ_H (500 MHz, Me_2CO-d_6) 0.49 and 0.90 (6H, 2d, 1:1, *J*=6.7 Hz, *Me*$_2$CH), 1.49 (9H, s, *t*-Bu), 1.68–1.83 (1H, m, C*H*Me$_2$), 2.79 (1H, ddd, *J*=4.8, 9.2, 12.9 Hz, 2-Ha), 3.02 (1H, ddd, *J*=9.2, 10.9, 12.9 Hz, 2-Hb), 3.36 (1H, t, *J*=10.9 Hz, 5-H), 4.56 (1H, dd, *J*=9.5, 10.9 Hz, 6-H), 4.69 (1H, dd, *J*=4.8, 10.9 Hz, 1-H), 4.79 (1H, t, *J*=9.2 Hz, 3-H), 5.09 and 5.17 (2H, 2d, 1:1, *J*=12.6 Hz, C*H*$_2$Ph), 6.85 (1H, d, *J*=9.5 Hz, NH), 7.28–7.38 (6H, m, Ph and *p*-C_6H_3), 7.43–7.49 (2H, m, *m*-C_6H_3); δ_C (126 MHz, $CDCl_3$) 19.5, 20.8, 28.1, 28.1, 36.8, 54.0, 55.2, 59.3, 67.5, 68.3, 82.5, 128.2, 128.3, 128.6, 128.9, 129.6, 131.0, 135.6, 136.1, 156.2, 168.8, 176.5; HRMS (ESI): MNa^+, found 584.1695. $C_{28}H_{33}Cl_2N_3NaO_5$ requires 584.1689.

*4.4.5. Synthesis of cycloadducts **13c** and **14c**.* Prepared from **5c** (3.65 g, 10 mmol), *tert*-butyl methacrylate (**6b**)(1.96 mL, 12 mmol), and anisole (40 mL), 7 h, MPLC (EtOAc/hexanes, 1:10).

4.4.5.1. tert-Butyl (1R,3R*,5R*,6R*)-6-(benzyloxycarbonylamino)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**13c**).* Yield: 1.35 g (35%) of white crystals; mp 133–136 °C; [Found: C, 68.68; H, 7.53; N, 8.28. $C_{29}H_{37}N_3O_5$ requires C, 68.62; H, 7.35; N, 8.28%]; ν_{max} (KBr) 3418, 2974, 2930, 1682, 1651, 1535, 1455, 1300, 1258, 1147, 1089, 1048, 881, 805, 750, 698, 668, 628 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.75 and 0.86 (6H, 2d, 1:1, *J*=6.7 Hz, *Me*$_2$CH), 1.32–1.40 (1H, m, Me$_2$C*H*), 1.52 (9H, s, *t*-Bu), 1.68 (3H, s, CH_3), 2.30 (1H, dd, *J*=11.3, 13.2 Hz, 2-Ha), 2.66 (1H, dd, *J*=5.7, 13.2 Hz, 2-Hb), 3.07 (1H, dd, *J*=2.9, 11.9 Hz, 5-H), 3.91 (1H, dd, *J*=5.7, 11.3 Hz, 3-H), 4.85 (1H, dd, *J*=11.9, 9.3 Hz, 6-H), 5.09 and 5.13 (2H, 2d, 1:1, *J*=12.3 Hz, C*H*$_2$Ph), 5.11 (1H, d, *J*=9.3 Hz, NH), 7.27–7.41 (10H, m, 2× Ph); δ_C (126 MHz, $CDCl_3$) 16.4, 18.5, 22.5, 27.4, 28.1, 52.9, 56.0, 62.6, 67.3, 70.1, 79.8, 82.9, 127.5, 128.3, 128.3, 128.6, 128.7, 128.8, 136.4, 138.6, 156.1, 163.3, 170.5; HRMS (ESI): MH^+, found 508.2808. $C_{29}H_{38}N_3O_5$ requires 508.2806.

4.4.5.2. tert-Butyl (1R,3S*,5R*,6R*)-6-(benzyloxycarbonylamino)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**14c**).* Yield: 0.45 g (9%) of white crystals; mp 95–98 °C; [Found: C, 68.24; H, 7.62; N, 8.06. $C_{29}H_{37}N_3O_5 \cdot {}^1/_3\ H_2O$ requires C, 67.82; H, 7.39; N, 8.18%]; ν_{max} (KBr) 3442, 3240, 3029, 2973, 1731, 1681, 1555, 1537, 1492, 1455, 1392, 1370, 1314, 1269, 1171, 1102, 1037, 985, 927, 853, 819, 743, 702 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.80 and 0.98 (6H, 2d, 1:1, *J*=6.8 Hz, *Me*$_2$CH), 1.52 (9H, s, *t*-Bu), 1.70 (3H, s, 1-Me), 1.80–1.89 (1H, m, Me$_2$C*H*), 2.65 (1H, dd, *J*=3.5, 8.1 Hz, 5-H), 2.70 (1H, dd, *J*=4.2, 13.5 Hz, 2-Ha), 2.78 (1H, dd, *J*=8.1, 13.5 Hz, 2-Hb), 4.39 (1H, dd, *J*=4.2, 8.1 Hz, 3-H), 4.63 (1H, t, *J*=8.7 Hz, 6-H), 5.07 (2H, s, C*H*$_2$Ph), 5.13 (1H, d, *J*=9.3 Hz, NH), 7.27–7.39 (8H, m, 8H of Ph), 7.44 (2H, d, *J*=7.2 Hz, 2H of Ph); δ_C (126 MHz, $CDCl_3$) 18.0, 19.0, 22.9, 27.9, 28.0, 50.8, 58.6, 60.8, 61.3, 67.2, 67.4, 83.0, 128.3, 128.3, 128.4, 128.6, 128.7, 128.8, 136.3, 138.2, 155.9, 160.5, 169.6; HRMS (ESI): MH^+, found 508.2799. $C_{29}H_{38}N_3O_5$ requires 508.2806.

4.4.5.3. Synthesis of tert-butyl (1R,3S*,5R*,6R*)-6-(benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-5-isopropyl-1-methyl-7-oxohexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**14d**).* Under argon, a mixture of **5d** (4.34 g, 10 mmol), *tert*-butyl methacrylate (1.96 mL, 12 mmol), and anisole (40 mL) was heated under reflux for 7 h. Volatile components were evaporated in vacuo, the residue was triturated with diethyl ether (50 mL), and the mixture was stirred at room temperature for 12 h. The precipitate was collected by filtration and washed with diethyl ether to give the first portion of **14d**. The filtrate was evaporated in vacuo and purified by FC (EtOAc). Fractions containing the product were combined and evaporated in vacuo to give the second portion of **14d**. Both portions of the product **14d** were combined. Yield: 3.80 g (66%) of white crystals; mp 161–165 °C; [Found C, 60.23; H, 6.22; N, 7.29. $C_{29}H_{35}Cl_2N_3O_5$ requires C, 60.42; H, 6.12; N, 7.29%]; ν_{max} (KBr) 3427, 3071, 3031, 2975, 2888, 1736, 1682, 1578, 1559, 1512, 1441, 1389, 1367, 1330, 1310, 1276, 1241, 1213, 1147, 1091, 1076, 1044, 966, 938, 914, 901, 847, 791, 757, 717, 726, 696, 667 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.91 and 1.09 (6H, 2d, 1:1, *J*=6.8 Hz, *Me*$_2$CH), 1.49 (9H, s, *t*-Bu), 1.82 (3H, s, 1-CH_3), 1.97–2.05 (1H, m, Me$_2$C*H*), 2.48 (1H, dd, *J*=7.7, 12.9 Hz, 2-Ha), 3.02 (1H, br t, *J*=2.3 Hz, 5-H), 3.16 (1H, dd, *J*=9.7, 12.9 Hz, 2-Hb), 4.61 (1H, dd, *J*=2.3, 9.2 Hz, 6-H), 5.09 in 5.14 (2H, 2d, *J*=12.2 Hz, C*H*$_2$Ph), 5.20 (1H, dd, *J*=7.7, 9.7 Hz, 3-H), 5.47 (1H, d, *J*=9.2 Hz, NH), 7.19 (1H, t, *J*=8.0 Hz, *p*-C_6H_3), 7.29–7.38 (7H, m, Ph and *m*-C_6H_3); δ_C (126 MHz, $CDCl_3$) 18.0, 20.3, 22.4, 27.3, 38.1, 45.8, 55.1, 59.3, 61.4, 67.0, 67.2, 82.7, 128.3, 128.3, 128.7, 129.1, 129.9, 131.3, 135.8, 136.3, 155.9, 161.9, 170.1; HRMS (ESI): MH^+, found 576.2054. $C_{29}H_{36}Cl_2N_3O_5$ requires 576.2027.

*4.4.6. General procedure for acidolytic deprotection of carboxy function. Synthesis of carboxylic acids **15a**,**c**, **16b**, **17b**,**d**, **18c**, **19d** and **31**.* A mixture of *tert*-butyl ester (0.5 mmol), dichloromethane (2 mL), and trifluoroacetic acid (1 mL) was stirred at room temperature for 24 h. Volatile components were evaporated in vacuo and the residue was triturated with ether (5 mL). The precipitate was collected by filtration and washed with ether to give the corresponding *C*-unprotected peptide (carboxylic acid).

The following compounds were prepared in this manner.

4.4.6.1. (1R,3R*,5R*,6R*)-6-(Benzyloxycarbonylamino)-7-oxo-3,5-diphenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylic acid (**15a**).* Prepared from **8a** (0.298 g, 0.5 mmol), trituration with ether. Yield: 0.235 g (100%) of white solid; mp 175–179 °C; [Found: C, 68.56; H, 5.54; N, 8.67. $C_{27}H_{25}N_3O_5$ requires C, 68.78; H, 5.34; N, 8.91%]; ν_{max} (KBr) 3331, 3064, 3035, 2893, 2619, 2502, 1751, 1734, 1653, 1537, 1496, 1457, 1449, 1292, 1273, 1261, 1242, 1134, 1066, 751, 696 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 2.57 (1H, dd, *J*=1.0, 5.4, 13.1 Hz, 2-Ha), 2.73 (1H, ddd, *J*=9.4, 11.4, 13.1 Hz, 2-Hb), 3.92 (1H, dd, *J*=5.4, 11.4 Hz, 3-H), 4.15 (1H, d, *J*=12.7 Hz, 5-H), 4.40 (1H, d, *J*=9.4 Hz, 1-H), 4.70 (1H, dd, *J*= 9.5, 12.7 Hz, 6-H), 4.90 and 4.97 (2H, 2d, 1:1, *J*=12.7 Hz, C*H*$_2$Ph), 6.98–7.34 (15H, m, 3×Ph), 8.04 (1H, d, *J*=9.5 Hz, NH), 13.25 (1H, br s, COOH); δ_C (126 MHz, $CDCl_3$) 42.4, 53.5, 63.0, 65.5, 68.4, 75.1, 127.6, 127.6, 127.6, 127.7, 127.7, 127.8, 127.8, 127.8, 128.3, 135.9, 136.7, 136.8, 155.9, 161.8, 170.6; HRMS (ESI): MH^-, found 470.1717. $C_{27}H_{24}N_3O_5$ requires 470.1716.

4.4.6.2. (1R,3R*,5R*,6R*)-6-(Benzyloxycarbonylamino)-5-isopropyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylic acid (**15c**).* Prepared from **8c** (0.335 g, 0.68 mmol), trituration with diisopropyl ether. Yield: 0.221 g (74%) of white solid; mp 197–200 °C; [Found: C, 65.69; H, 6.15; N, 9.30. $C_{24}H_{27}N_3O_5$ requires C, 65.89; H, 6.22; N, 9.60%]; ν_{max} (KBr) 3461, 2972, 1748, 1718, 1653, 1545, 1496, 1457, 1292, 1248, 1141, 1056, 765, 746, 693 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.75 and 0.87 (6H, 2d, 1:1, *J*=6.8 Hz, *Me*$_2$CH), 1.34–1.41 (1H, m, C*H*Me$_2$), 2.62–2.72 (2H, m, 2-CH_2), 3.25 (1H, dd, *J*=2.8, 12.4 Hz, 5-H), 4.06 (1H, t, *J*=8.4, 3-H), 4.41(1H, br t, *J*=5.1 Hz, 1-H), 5.10 and 5.15 (2H, 2d, 1:1, *J*=12.7 Hz, C*H*$_2$Ph), 5.12 (1H, m, 6-H, overlapped by the signal for CH_2Ph), 6.65 (1H, d, *J*=9.6 Hz, NH),

6.64–7.38 (10H, m, 2× Ph), 7.78 (1H, br s, COOH); δ_C (126 MHz, $CDCl_3$) 16.3, 18.2, 27.1, 43.2, 55.3, 56.4, 66.9, 69.2, 80.8, 127.5, 127.7, 128.0, 128.5, 128.8, 129.0, 136.7, 137.6, 156.5, 165.4, 170.7; HRMS (ESI): MH^+, found 438.2022. $C_{24}H_{28}N_3O_5$ requires 438.2023.

4.4.6.3. (1S,3S*,5R*,6R*)-6-(Benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-7-oxo-5-phenyl-hexahydropyrazolo[1,2-a]pyrazole-1-carboxylic acid (**16b**).* Prepared from **9b** (0.298 g, 0.5 mmol), trituration with diisopropyl ether. Yield: 0.269 g (100%) of yellowish solid; mp 116–120 °C; [Found: C, 59.74; H, 3.96; N, 7.89. $C_{27}H_{23}Cl_2N_3O_5$ requires C, 60.01; H, 4.29; N, 7.78%]; ν_{max} (KBr) 3481, 3302, 3067, 2953, 1722 (C=O), 1683 (C=O), 1578, 1555, 1456, 1439, 1344, 1288, 1253, 1233, 1210, 1160, 1058, 1004, 786, 779, 762, 697 cm^{-1}; δ_H (500 MHz, DMSO-d_6) 2.96 (1H, ddd, *J*=4.1, 9.8, 14.2 Hz, 2-Ha), 3.21 (1H, ddd, *J*=4.3, 10.4, 14.2 Hz, 2-Hb), 3.82 (1H, d, *J*=12.1 Hz, 5-H), 4.53 (1H, dd, *J*=9.2, 12.1 Hz, 6-H), 4.85 (1H, dd, *J*=4.1, 10.4 Hz, 3-H), 4.91 and 4.96 (2H, 2d, 1:1, *J*=12.5 Hz, CH_2Ph), 5.29 (1H, dd, *J*=4.3, 9.8 Hz, 1-H), 6.99–7.63 (13H, m, 2× Ph and C_6H_3), 8.03 (1H, d, *J*=9.2 Hz, NH), 13.27 (1H, br s, COOH); δ_C (126 MHz, DMSO-d_6) 35.1, 52.8, 57.6, 62.9, 65.5, 67.1, 127.4, 127.6, 127.8, 128.2, 128.3, 128.3, 128.9, 130.5, 130.8, 131.6, 134.7, 136.7, 155.9, 160.4, 171.0; HRMS (ESI): MH^+, found 540.1084. $C_{27}H_{24}Cl_2N_3O_5$ requires 540.1093.

4.4.6.4. (2S,3S*,5R*,6R*)-6-(Benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-7-oxo-5-phenylhexahydropyrazolo[1,2-a]pyrazole-2-carboxylic acid (**17b**).* Prepared from **11b** (4.47 g, 7.5 mmol), trituration with ether. Yield: 3.43 g (84%) of white solid; mp 185–192 °C; [Found: C, 59.83; H, 4.25; N, 7.81. $C_{27}H_{23}Cl_2N_3O_5$ requires C, 60.01; H, 4.29; N, 7.78%]; ν_{max} (KBr) 3248, 3039, 1729 (C=O), 1686 (C=O), 1561, 1537, 1486, 1456, 1437, 1425, 1321, 1278, 1243, 1225, 1187, 1052, 1031, 784, 698, 480 cm^{-1}; δ_H (500 MHz, DMSO-d_6) 3.49 (1H, ddd, *J*=3.6, 7.5, 9.3 Hz, 2-H), 3.71 (1H, dd, *J*=9.3, 11.7 Hz, 1-Ha), 4.07 (1H, dd, *J*=3.6, 11.7 Hz, 1-Hb), 4.68 (1H, d, *J*=7.5 Hz, 3-H), 4.83 (1H, d, *J*=12.1 Hz, 5-H), 5.02 (1H, dd, *J*=9.0, 12.1 Hz, 6-H), 5.06 (2H, s, CH_2Ph), 7.48–6.72 (13H, m, 2× Ph and C_6H_3), 7.96 (1H, d, *J*=9.0 Hz, NH), 12.90 (1H, s, COOH); δ_C (126 MHz, DMSO-d_6) 45.8, 51.1, 51.3, 61.8, 62.3, 66.0, 127.7, 127.8, 127.9, 128.0, 128.4, 129.7, 130.2, 131.3, 132.5, 134.2, 135.3, 136.6, 156.1, 173.5, 175.2; HRMS (ESI): MH^+, found 540.1072. $C_{27}H_{24}Cl_2N_3O_5$ requires 540.1093.

4.4.6.5. (2S,3S*,5R*,6R*)-6-(Benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-5-isopropyl-7-oxohexahydropyrazolo[1,2-a]pyrazole-2-carboxylic acid (**17d**).* Prepared from **11d** (510 mg, 0.9 mmol), trituration with diisopropyl ether. Yield: 347 mg (76%) of white solid; mp 181–185 °C; [Found: C, 56.86, H, 4.90; N, 8.17. $C_{24}H_{25}Cl_2N_3O_5$ requires C, 56.93; H, 4.98; N, 8.30%]; ν_{max} (KBr) 3414, 3302, 3065, 3034, 2966, 2933, 2878, 1741, 1720, 1661, 1654, 1579, 1561, 1533, 1498, 1456, 1441, 1391, 1295, 1266, 1245, 1212, 1151, 1086, 1055, 1028, 778, 700, 641 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.70 and 0.94 (6H, 2d, 1:1, *J*=6.8 Hz, *Me*$_2$CH), 1.76–1.90 (1H, m, C*H*Me$_2$), 2.84 (1H, dd, *J*=6.2, 9.9 Hz, 5-H), 3.81 (1H, dd, *J*=6.4, 11.8 Hz, 1-Ha), 3.89 (1H, td, *J*=6.1, 9.5 Hz, 2-H), 4.22 (1H, dd, *J*=9.5, 11.8 Hz, 1-Hb), 4.82 (1H, t, *J*=9.9 Hz, 6-H), 5.07 and 5.14 (2H, 2d, 1:1, *J*=12.1 Hz, CH_2Ph), 5.29 (1H, d, *J*=9.9 Hz, NH), 5.66 (1H, d, *J*=5.8 Hz, 3-H), 6.07 (1H, br s, COOH), 7.13–7.47 (8H, m, Ph and C_6H_3); δ_C (126 MHz, $CDCl_3$) 18.5, 19.2, 29.9, 43.2, 49.7, 56.5, 62.6, 67.6, 69.8, 128.4, 128.5, 128.7, 129.9, 130.6, 130.7, 134.7, 137.9, 156.1, 166.7, 174.5; HRMS (ESI): MNH_4^+, found 523.1504. $C_{24}H_{29}Cl_2N_4O_5$ requires 523.151.

4.4.6.6. (1R,3R*,5R*,6R*)-6-(Benzyloxycarbonylamino)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylic acid (**18c**).* Prepared from **13c** (2.58 g, 5 mmol), trituration with ether. Yield: 3.39 g (75%) of white crystals; mp 204–205 °C; [Found: C, 66.06; H, 6.54; N, 9.34. $C_{25}H_{29}N_3O_5 \cdot {}^1/_{10}H_2O$ requires C, 66.24; H, 6.49; N, 9.27%]; ν_{max} (KBr) 3297, 3054, 2938, 2635, 1746, 1731, 1659, 1651, 1538, 1495, 1469, 1455, 1437, 1377, 1322, 1296, 1248, 1208, 1179, 1147, 1110, 1061, 1021, 1000, 967, 943, 858, 836, 814, 782, 749, 739, 669, 629 cm^{-1}; δ_H (500 MHz, DMSO-d_6) 0.62 and 0.71 (6H, 2d, 1:1, *J*=7.0 Hz, *Me*$_2$CH), 1.15–1.25 (1H, m, Me$_2$C*H*), 1.54 (3H, s, 1-Me), 2.26 (1H, dd, *J*=10.9, 13.2 Hz, 2-Ha), 2.78 (1H, dd, *J*=5.8, 13.2 Hz, 2-Hb), 3.17 (1H, dd, *J*=3.0, 12.1 Hz, 5-H), 3.94 (1H, dd, *J*=5.8, 10.9 Hz, 3-H), 4.53 (1H, dd, *J*=9.6, 12.1 Hz, 6-H), 5.06 (2H, s, CH_2Ph), 7.29–7.38 (8H, m, 8H of Ph), 7.46 (2H, d, *J*=7.3 Hz, 2H of Ph), 7.94 (1H, d, *J*=9.6 Hz, NH), 13.13 (1H, s, COOH); δ_C (126 MHz, DMSO-d_6) 16.1, 18.4, 22.3, 26.8, 51.8, 55.1, 60.1, 65.5, 69.5, 75.1, 127.58, 127.63, 127.8, 128.3, 128.4, 128.5, 137.1, 138.5, 155.8, 161.6, 172.1; HRMS (ESI): MH^-, found 450.2035. $C_{25}H_{28}N_3O_5$ requires 450.2034.

4.4.6.7. (1R,3S*,5R*,6R*)-6-(Benzyloxycarbonylamino)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylic acid (**19d**).* Prepared from **14d** (2.88 g, 5 mmol), trituration with ether. Yield: 3.52 g (67%) of white crystals; mp 159–160 °C; ν_{max} (KBr) 3551, 3447, 3262, 3067, 2963, 2933, 2878, 1731, 1698, 1633, 1581, 1548, 1497, 1438, 1379, 1336, 1304, 1258, 1222, 1190, 1175, 1148, 1133, 1065, 1046, 970, 933, 874, 804, 776, 761, 730, 719, 691, 659 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.75 and 1.01 (6H, 2d, 1:1, *J*=6.9 Hz, *Me*$_2$CH), 1.74–1.81 (1H, m, Me$_2$C*H*), 1.85 (3H, s, 1-Me), 2.79 (1H, dd, *J*=3.5, 10.1 Hz, 5-H), 2.91 (1H, dd, *J*=10.1, 14.5 Hz, 2-Ha), 3.37 (1H, dd, *J*=4.9, 14.5 Hz, 2-Hb), 5.05 (1H, t, *J*=10.1 Hz, 6-H), 5.10 and 5.15 (2H, 2d, 1:1, *J*=12.1 Hz, CH_2Ph), 5.58 (1H, dd, *J*=4.9, 10.1 Hz, 3-H), 7.24 (1H, t, *J*=8.0 Hz, *p*-C_6H_3), 7.29–7.42 (7H, m, Ph and *p*-C_6H_3), NH and COOH exchanged; δ_C (126 MHz, $CDCl_3$) 16.8, 18.5, 25.3, 28.1, 44.8, 55.5, 57.4, 65.9, 67.6, 69.1, 128.2, 128.4, 128.6, 129.7, 130.5, 130.7, 132.0, 134.1, 135.7, 137.4, 155.7, 161.8, 171.5; HRMS (ESI): MH^+, found 520.1428. $C_{25}H_{28}Cl_2N_3O_5$ requires 520.1401.

4.4.6.8. (1R,3R*,5R*,6R*)-6-(2-(2-(Benzyloxycarbonylamino)acetamido)acetamido)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylic acid (**31**).* Prepared from the ester **30** (311 mg, 0.5 mmol). Yield: 65 mg (23%) of white crystals; mp 128–129 °C; [Found: C, 57.58; H, 6.20; N, 11.52. $C_{29}H_{35}N_5O_7 \cdot 2H_2O$ requires C, 57.89; H, 6.53; N, 11.64%]; ν_{max} (KBr) 3459, 3069, 2964, 1674, 1542, 1457, 1439, 1374, 1264, 1217, 1150, 1087, 1051, 1029, 990, 974, 909, 858, 745, 699, 675, 653, 633, 609 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.68 and 0.83 (6H, 2d, 1:1, *J*=6.8 Hz, *Me*$_2$CH), 1.25–1.31 (1H, m, Me$_2$C*H*), 1.70 (3H, s, CH_3), 2.30 (1H, br dd, *J*=11.5, 13.2 Hz, 2-Ha), 2.87 (1H, br dd, *J*=5.8, 13.2 Hz, 2-Hb), 3.35 (1H, br d, *J*=12.0 Hz, 5-H), 3.84–4.12 (5H, m, 3-H and 2×CH_2NH), 5.05 and 5.13 (2H, 2d, 1:1, *J*=12.4 Hz, CH_2Ph), 5.10–5.18 (1H, m, 6-H, overlapped by the signals for CH_2Ph), 6.19 (1H, br s, N*H*CH$_2$), 7.24–7.38 (10H, m, 2× Ph), 7.76 (1H, br s, N*H*CH$_2$), 8.16 (1H, br d, *J*=6.0 Hz, N*H*CH), COOH exchanged; δ_C (126 MHz, $CDCl_3$) 16.3, 18.6, 22.5, 27.5, 42.8, 44.6, 52.5, 54.2, 61.7, 67.3, 70.3, 77.4, 127.6, 128.1, 128.3, 128.7, 128.8, 128.9, 136.4, 137.8, 157.3, 162.9, 170.2, 171.1, 173.0; HRMS (ESI): MH^+, found 566.2609. $C_{29}H_{36}N_5O_7$ requires 566.2609.

*4.4.7. General procedure for hydrogenolytic deprotection of amino function. Synthesis of amines **20a**,**c**, **21b**,**d**, **22c**, **23d** and **26**.* A mixture of a Cbz-protected peptide (1 mmol), ethanol (40 mL), and 10% Pd–C (30 mg) was hydrogenated (3.5 bar of H_2) at rt for 3–6 h. The catalyst was removed by filtration through a fritted funnel, washed with ethanol (2×5 mL), and the combined filtrate was evaporated in vacuo. The residue was purified by FC (EtOAc). Fractions containing the product were combined and evaporated in vacuo to give the *N*-unprotected peptide (amine).

The following compounds were prepared in this manner.

4.4.7.1. tert-Butyl (1R,3R*,5R*,6R*)-6-amino-7-oxo-3,5-diphenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**20a**).* Prepared from **8a** (0.298 g, 0.5 mmol). Yield: 40 mg (20%) of white solid; mp 114–119 °C; [Found: C, 69.61; H, 6.77; N, 10.26. $C_{23}H_{27}N_3O_3 \cdot {}^1/_4H_2O$

requires C, 69.41; H, 6.96; N, 10.56%]; ν_{max} (KBr) 3440, 3372, 2980, 1728, 1704, 1456, 1426, 1376, 1366, 1358, 1229, 1152, 1140, 1125, 696 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.56 (9H, s, *t*-Bu), 1.61 (2H, s, NH_2), 2.52 (1H, ddd, *J*=1.2, 5.5, 13.2 Hz, 2-Ha), 2.78 (1H, ddd, *J*=9.2, 11.6, 13.2 Hz, 2-Hb), 3.87 (1H, d, *J*=11.8 Hz, 5-H), 3.96 (1H, dd, *J*=5.5, 11.6 Hz, 3-H), 4.00 (1H, br d, *J*=11.8 Hz, 6-H), 4.41 (1H, br d, *J*=9.2 Hz, 1-H), 7.00–7.06, 7.10–7.14, and 7.16–7.19 (10H, 3m, 6:2:2, 2× Ph); δ_C (126 MHz, $CDCl_3$) 28.2, 43.1, 55.0, 65.0, 69.0, 81.1, 83.2, 127.8, 127.9, 128.0, 128.1, 128.1, 128.1, 136.4, 136.5, 166.4, 169.0; HRMS (ESI): MH^+, found 394.2131. $C_{23}H_{28}N_3O_3$ requires 394.2125.

4.4.7.2. tert-Butyl (1R,3R*,5R*,6R*)-6-amino-5-isopropyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**20c**).* Prepared from **8c** (0.300 g, 0.61 mmol). Yield: 0.134 g (62%) of purplish oil; [Found: C, 66.67; H, 8.08; N, 11.55. $C_{20}H_{29}N_3O_3$ requires C, 66.83; H, 8.13; N, 11.69%]; ν_{max} (liquid film) 3462, 2980, 2960, 1733, 1686, 1457, 1420, 1368, 1225, 1155, 1106, 852, 745, 698 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.77 and 0.85 (6H, 2d, 1:1, *J*=7.0 Hz, Me_2CH), 1.39 (1H, doublet of quintet, *J*=3.1, 6.9 Hz, Me_2CH), 1.53 (9H, s, *t*-Bu), 1.62 (2H, br s, NH_2), 2.50 (1H, ddd, *J*=1.2, 5.5, 13.2 Hz, 2-Ha), 2.66 (1H, ddd, *J*=9.3, 11.4, 13.2 Hz, 2-Hb), 2.98 (1H, dd, *J*=3.1, 11.3 Hz, 5-H), 3.83 (1H, br d, *J*=11.3 Hz, 6-H), 3.88 (1H, dd, *J*=5.5, 11.4 Hz, 3-H), 4.28 (1H, br d, *J*=9.3 Hz, 1-H), 7.30–7.41 (5H, m, Ph); δ_C (126 MHz, $CDCl_3$) 16.8, 19.4, 27.7, 28.1, 44.1, 55.0, 57.4, 69.2, 80.8, 83.0, 127.6, 128.6, 128.8, 138.3, 167.9, 169.0; HRMS (ESI): MH^+, found 360.2283. $C_{20}H_{30}N_3O_3$ requires 360.2282.

4.4.7.3. tert-Butyl (2S,3S*,5R*,6R*)-6-amino-3-(2,6-dichlorophenyl)-7-oxo-5-phenylhexahydropyrazolo[1,2-a]pyrazole-2-carboxylate (**21b**).* Prepared from **11b** (4.77 g, 8 mmol). Yield: 2.43 g (66%) of white solid; mp 138–143 °C; ν_{max} (KBr) 3397, 3331, 3067, 3003, 2978, 2956, 2932, 2886, 1734(C=O), 1713, 1582, 1561, 1452, 1440, 1380, 1367, 1353, 1324, 1267, 1239, 1153, 1086, 839, 786, 772, 757, 703 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.40 (9H, s, *t*-Bu), 1.67 (2H, br s, NH_2), 3.76 (1H, td, *J*=6.6, 7.8 Hz, 2-H), 4.02 (2H, d, *J*=7.8 Hz, 1-CH_2), 4.12 (1H, d, *J*=11.1 Hz, 6-H), 4.23 (1H, d, *J*=11.1 Hz, 5-H), 5.09 (1H, d, *J*=6.6 Hz, 3-H), 6.90–7.37 (8H, m, Ph and C_6H_3); δ_C (126 MHz, $CDCl_3$) 27.9, 44.5, 51.6, 57.5, 61.9, 68.9, 82.4, 127.9, 128.3, 128.3, 128.6, 129.5, 130.2, 132.8, 135.0, 171.0, 172.7; HRMS (ESI): MH^+, found 462.1355. $C_{23}H_{26}Cl_2N_3O_3$ requires 462.1351.

4.4.7.4. tert-Butyl (2S,3S*,5R*,6R*)-6-amino-3-(2,6-dichlorophenyl)-5-isopropyl-7-oxohexahydropyrazolo[1,2-a]pyrazole-2-carboxylate (**21d**).* Prepared from **11d** (558 mg, 1 mmol). Yield: 324 mg (76%) of purple oil; ν_{max} (liquid film) 3386, 3307, 2974, 2930, 2873, 1730, 1682, 1580, 1561, 1458, 1438, 1390, 1368, 1272, 1256, 1215, 1154, 1085, 1034, 840, 783, 772, 740, 666 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.71 and 0.98 (6H, 2d, 1:1, *J*=6.7 Hz, $CHMe_2$), 1.46 (9H, s, *t*-Bu), 1.70 (2H, br s, NH_2), 1.81 (1H, sextet, *J*=6.7 Hz, $CHMe_2$), 2.75 (1H, dd, *J*=7.5, 9.6 Hz, 5-H), 3.67 (1H, d, *J*=9.6 Hz, 6-H), 3.75 (1H, td, *J*=5.8, 9.5 Hz, 2-H), 3.84 (1H, dd, *J*=5.8, 11.7 Hz, 1-Ha), 4.10 (1H, dd, *J*=9.5, 11.7 Hz, 1-Hb), 5.42 (1H, d, *J*=5.8 Hz, 3-H), 7.24 (1H, t, *J*=8.0 Hz, *p*-C_6H_3), 7.40 (2H, dt, *J*=1.0, 7.8 Hz, *m*-C_6H_3); δ_C (126 MHz, $CDCl_3$) 19.2, 20.0, 28.0, 30.2, 43.6, 51.6, 57.0, 62.8, 71.7, 82.5, 129.5, 130.1, 130.7, 133.6, 170.9, 171.8; HRMS (ESI): MH^+, found 428.1499. $C_{20}H_{28}Cl_2N_3O_3$ requires 428.1502.

4.4.7.5. tert-Butyl (1R,3R*,5R*,6R*)-6-amino-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**22c**).* Prepared from **13c** (2.54 g, 5 mmol). Yield: 2.58 g (69%) of reddish oil; ν_{max} (liquid film) 3432, 2970, 2925, 2868, 1734, 1684, 1457, 1434, 1390, 1369, 1301, 1253, 1212, 1158, 1070, 974, 847, 751, 701, 607 cm^{-1}; NMR (500 MHz, $CDCl_3$) 0.78 and 0.84 (6H, 2d, 1:1, *J*=6.8 Hz, Me_2CH), 1.31–1.42 (1H, m, Me_2CH), 1.54 (9H, s, *t*-Bu), 1.62 (2H, s, NH_2), 1.67 (3H, s, 1-Me), 2.30 (1H, dd, *J*=11.2, 13.2 Hz, 2-Ha), 2.69 (1H, dd, *J*=5.8, 13.2 Hz, 2-Hb), 2.93 (1H, dd, *J*=3.1, 11.0 Hz, 5-H), 3.77 (1H, d, *J*=11.0 Hz, 6-H), 3.92 (1H, dd, *J*=5.8, 11.2 Hz, 3-H), 7.28–7.37 (3H, m, *o*,*p*-Ph), 7.37–7.41 (2H, m, *m*-Ph); δ_C (126 MHz, $CDCl_3$) 16.7, 19.3, 22.5, 27.7, 28.0, 52.9, 56.9, 61.8, 70.4, 80.5, 82.5, 127.5, 128.3, 128.6, 138.5, 166.7, 170.4; HRMS (ESI): MH^+, found 374.2439. $C_{21}H_{32}N_3O_3$ requires 374.2438.

4.4.7.6. tert-Butyl (1R,3S*,5R*,6R*)-6-amino-3-(2,6-dichlorophenyl)-5-isopropyl-1-methyl-7-oxohexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**23d**).* Prepared from **14d** (2.88 g, 5 mmol). Yield: 0.7 g (93%) of red crystals; mp 109–112 °C; [Found: C, 53.73; H, 6.87; N, 8.95. $C_{21}H_{29}Cl_2N_3O_3 \cdot 1½H_2O$ requires C, 53.38; H, 6.71; N, 8.93%]; ν_{max} (KBr) 3448, 2969, 2930, 1733, 1699, 1560, 1522, 1454, 1438, 1394, 1369, 1287, 1257, 1204, 1150, 1124, 1085, 930, 845, 788, 757, 701, 648, 618 cm^{-1}; NMR (500 MHz, $CDCl_3$) 0.95 and 1.11 (6H, 2d, 1:1, *J*=6.6 Hz, Me_2CH), 1.28 (1H, d, *J*=8.6 Hz, Me_2CH), 1.51 (9H, s, *t*-Bu), 1.84 (3H, s, 1-Me), 2.08 (2H, br s, NH_2), 2.53 (1H, dd, *J*=7.7, 12.9 Hz, 2-Ha), 3.20 (1H, dd, *J*=9.9, 12.9 Hz, 2-Hb), 3.42 (1H, br s, 5-H), 4.20 (1H, br s, 6-H), 5.22 (1H, br t, *J*=8.7 Hz, 3-H), 7.21 (1H, t, *J*=8.0 Hz, *p*-C_6H_3), 7.34 (2H, br t, *J*=7.8 Hz, *m*-C_6H_3); δ_C (126 MHz, $CDCl_3$) 18.1, 20.3, 22.3, 27.3, 28.1, 45.8, 54.9, 58.6, 61.7, 65.0, 83.3, 129.0, 130.1, 131.5, 136.1, 161.9, 170.4; HRMS (ESI): MH^+, found 442.1677. $C_{21}H_{30}Cl_2N_3O_3$ requires 442.1659.

4.4.7.7. Methyl 2-((1R,3R*,5R*,6R*)-6-amino-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)acetate (**26**).* Prepared from *N*-protected tripeptide **25** (627 mg, 1.19 mmol). Yield: 462 mg (100%) of red oil; ν_{max} (liquid film) 3488, 3416, 3363, 2956, 2873, 1750, 1674, 1525, 1438, 1369, 1307, 1208, 1145, 1021, 976, 902, 873, 748, 701, 671, 656 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.77 and 0.84 (6H, 2d, 1:1, *J*=7.0 Hz, Me_2CH), 1.29 (1H, doublet of quintet, *J*=2.8, 7.0 Hz, Me_2CH), 1.66 (2H, br s, NH_2), 1.80 (3H, s, CH_3), 2.22 (1H, dd, *J*=11.2, 12.7 Hz, 2-Ha), 2.85 (1H, dd, *J*=2.8, 11.3 Hz, 5-H), 3.48 (1H, dd, *J*=5.1, 12.7 Hz, 2-Hb), 3.60 (1H, dd, *J*=5.1, 11.2 Hz, 3-H), 3.76 (3H, s, OMe), 3.86 (1H, d, *J*=11.3 Hz, 6-H), 3.98 (1H, dd, *J*=5.1 17.9 Hz, 1H of CH_2NH), 4.15 (1H, dd, *J*=6.2, 17.9 Hz, 1H of CH_2NH), 7.28–7.41 (5H, m, Ph), 8.95 (1H, t, *J*=5.1 Hz, N*H*CH_2); δ_C (126 MHz, $CDCl_3$) 16.7, 19.7, 23.7, 26.8, 41.8, 51.2, 52.5, 58.0, 64.9, 69.0, 79.3, 127.7, 128.6, 128.7, 138.0, 167.3, 170.4, 172.7; HRMS (ESI): MH^+, found 389.2187. $C_{20}H_{29}N_4O_4$ requires 389.2183.

*4.4.8. General procedure for the synthesis of the racemic tripeptide **25**, tetrapeptides **28** and **30**, and hexapeptide **33**.* 4-Methylmorpholine (0.11 mL, 1 mmol) and EEDQ (247 mg, 1 mmol) were added to a stirred mixture of carboxylic acid (1 mmol), amine hydrochloride (1 mmol), and anhydrous dichloromethane (5 mL) and the mixture was stirred at room temperature for 24 h (Addition of 4-methylmorpholine was omitted when free amine was used.) The volatile components were evaporated in vacuo. The residue was dissolved in EtOAc (5 mL) and then washed, subsequently, with 1 M aq $NaHSO_4$ (5 mL) and aq $NaHCO_3$ (5 mL), dried over Na_2SO_4, filtered, and the filtrate vas evaporated in vacuo. The residue was purified by FC (EtOAc). Fractions containing the product were combined and evaporated in vacuo to give the corresponding carboxamide.

The following compounds were prepared in this manner.

4.4.8.1. Methyl 2-((1R,3R*,5R*,6R*)-6-(benzyloxycarbonylamino)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)acetate (**25**).* Prepared from carboxylic acid **18c** (451 mg, 1.0 mmol), methyl glycinate hydrochloride (**24**)(126 mg, 1.0 mmol), 4-methylmorpholine (0.11 mL, 1 mmol), and EEDQ (247 mg, 1.0 mmol). Yield: 259 mg (50%) of white crystals; mp 171–173 °C; ν_{max} (KBr) 3457, 3362, 3245, 3035, 2934, 2850, 1758, 1687, 1672, 1628, 1537, 1496, 1457, 1435, 1402, 1388, 1366, 1275, 1202, 1179, 1124, 1093, 1056, 1026, 978, 963, 917, 858, 843, 813, 785, 747, 700, 664, 626 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.73 and 0.82 (6H, 2d, 1:1, *J*=6.9 Hz, Me_2CH), 1.23–1.38 (1H, m, Me_2CH), 1.79 (3H, s, CH_3), 2.29

(1H, dd, *J*=11.2, 12.8 Hz, 2-Ha), 3.24 (1H, dd, *J*=5.2, 12.8 Hz, 2-Hb), 3.31 (1H, dd, *J*=3.1, 11.4 Hz, 5-H), 3.75 (3H, s, OMe), 3.78 and 4.29 (2H, 2dd, 1:1, *J*=5.9, 17.7 Hz, C*H*$_2$NH), 3.83 (1H, dd, *J*=5.2, 11.2 Hz, 3-H), 4.56 (1H, dd, *J*=8.7, 11.4 Hz, 6-H), 5.08 and 5.12 (2H, 2d, 1:1, *J*=12.3 Hz, C*H*$_2$Ph), 5.65 (1H, d, *J*=8.7 Hz, N*H*CH), 7.29–7.41 (10H, m, 2× Ph), 8.56 (1H, t, *J*=5.6 Hz, N*H*CH$_2$); δ_C (126 MHz, DMSO-d_6) 16.2, 19.1, 22.9, 27.0, 34.1, 41.7, 52.2, 52.4, 57.3, 67.4, 68.9, 75.8, 127.7, 128.1, 128.4, 128.6, 128.7, 128.8, 136.2, 137.8, 156.2, 162.6, 170.7, 172.3; HRMS (ESI): MH$^+$, found 523.2545. $C_{28}H_{35}N_4O_6$ requires 523.2551.

4.4.8.2. Methyl 2-((1R,3R*,5R*,6R*)-6-(2-(tert-butoxycarbonylamino)acetamido)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)acetate (**28**).* Prepared from amine **26** (370 mg, 0.95 mmol), Boc-glycine (**27**)(175 mg, 1 mmol), 4-methylmorpholine (0.11 mL, 1 mmol), and EEDQ (247 mg, 1.0 mmol). Yield: 517 mg (87%) of red crystals; 104–108 °C; ν_{max} (KBr) 3491, 3426, 3069, 2964, 2875, 1755, 1682, 1538, 1456, 1438, 1392, 1367, 1249, 1206, 1172, 1050, 1027, 980, 946, 862, 748, 702, 667, 630 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.70 and 0.81 (6H, 2d, 1:1, *J*=6.8 Hz, *Me*$_2$CH), 1.20–1.28 (1H, m, Me$_2$C*H*), 1.43 (9H, s, Boc), 1.79 (3H, s, CH$_3$), 2.32 (1H, dd, *J*=11.2, 13.0 Hz, 2-Ha), 3.17 (1H, dd, *J*=5.1, 13.0 Hz, 2-Hb), 3.42 (1H, dd, *J*=3.3, 11.2 Hz, 5-H), 3.74 (1H, dd, *J*=5.7, 16.8 Hz, 1H of C*H*$_2$NH), 3.78 (3H, s, OMe), 3.83 (1H, dd, *J*=6.2, 16.8 Hz, 1H of C*H*$_2$NH), 3.85 (1H, dd, *J*=5.2, 17.4 Hz, 1H of C*H*$_2$NH), 3.95 (1H, dd, *J*=5.1, 11.2 Hz, 3-H), 4.32 (1H, dd, *J*=6.6, 17.4 Hz, 1H of C*H*$_2$NH), 4.61 (1H, br t, *J*=9.4 Hz, 6-H), 5.42 (1H, t, *J*=5.9 Hz, N*H*CH$_2$), 7.29–7.35 (3H, m, *o*,*p*-Ph), 7.37–7.41 (2H, m, *m*-Ph), 7.48 (1H, br d, *J*=6.6 Hz, N*H*CH), 8.58 (1H, t, *J*=5.7 Hz, N*H*CH$_2$); δ_C (126 MHz, $CDCl_3$) 16.0, 19.2, 22.7, 27.2, 28.5, 41.8, 44.5, 52.5, 52.9, 56.3, 63.8, 68.8, 74.2, 80.4, 127.7, 128.7, 128.8, 137.6, 156.3, 161.7, 170.6, 170.8, 172.1; HRMS (ESI): MH$^+$, found 546.2917. $C_{27}H_{40}N_5O_7$ requires 546.2922.

4.4.8.3. tert-Butyl (1R,3R*,5R*,6R*)-6-(2-(2-(benzyloxycarbonylamino)acetamido)acetamido)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**30**).* Prepared from amine **22c** (374 mg, 1 mmol), *N*-(Z-glycyl)glycine (**29**)(266 mg, 1 mmol), 4-methylmorpholine (0.11 mL, 1 mmol), and EEDQ (246 mg, 1 mmol). Yield: 466 mg (74%) of pink crystals; mp 90–94 °C; [Found: C, 63.05; H, 7.23; N, 10.73. $C_{33}H_{43}N_5O_7 \cdot ½H_2O$ requires C, 62.84; H, 7.03; N, 11.10%]; ν_{max} (KBr) 3432, 3067, 2973, 2925, 2873, 1731, 1693, 1681, 1651, 1537, 1455, 1393, 1369, 1257, 1148, 1085, 1048, 1028, 988, 915, 885, 844, 745, 698, 651, 611 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.70 and 0.81 (6H, 2d, 1:1, *J*=6.6 Hz, *Me*$_2$CH), 1.24–1.30 (1H, m, Me$_2$C*H*), 1.48 (9H, s, *t*-Bu), 1.64 (3H, s, CH$_3$), 2.28 (1H, dd, *J*=11.2, 13.3 Hz, 2-Ha), 2.67 (1H, dd, *J*=5.4, 13.3 Hz, 2-Hb), 3.25 (1H, dd, *J*=3.2, 11.8 Hz, 5-H), 3.77 (1H, dd, *J*=5.3, 16.5 Hz, 1H of C*H*$_2$NH), 3.83 (1H, dd, *J*=5.4, 17.1 Hz, 1H of C*H*$_2$NH), 3.91 (1H, dd, *J*=5.4, 11.2 Hz, 3-H), 3.93 (1H, dd, *J*=5.8, 17.7 Hz, 1H of C*H*$_2$NH), 4.09 (1H, dd, *J*=5.9, 17.7 Hz, 1H of C*H*$_2$NH), 5.11 (1H, dd, *J*=7.2, 11.8 Hz, 6-H), 5.11 and 5.15 (2H, 2d, 1:1, *J*=12.4 Hz, C*H*$_2$Ph), 6.16 (1H, t, *J*=5.8 Hz, N*H*CH$_2$), 7.27–7.39 (10H, m, 2× Ph), 7.62–7.70 (2H, m, N*H*CH and N*H*CH$_2$); δ_C (126 MHz, $CDCl_3$) 15.3, 16.2, 18.4, 22.3, 27.3, 42.7, 44.4, 52.3, 54.0, 61.5, 65.9, 67.1, 70.1, 127.4, 128.0, 128.1, 128.5, 128.7, 136.2, 137.6, 157.2, 162.7, 169.9, 170.1, 170.8, 171.0, 172.8; HRMS (ESI): MH$^+$, found 622.3241. $C_{33}H_{44}N_5O_7$ requires 622.3235.

4.4.8.4. Methyl 2-(2-((1R,3R*,5R*,6R*)-6-(2-(2-(benzyloxycarbonylamino)acetamido)acetamido)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)acetamido)acetate (**33**).* Prepared from carboxylic acid **31** (175 mg, 0.3 mmol), methyl *N*-(glycyl)glycinate hydrochloride (**32**)(56 mg, 0.3 mmol), 4-methylmorpholine (0.033 mL, 0.3 mmol), and EEDQ (76 mg, 0.3 mmol); General procedure A. Yield: 135 mg (62%) of red crystals; mp 114–117 °C; [Found: C, 56.60; H, 6.42; N, 13.14. $C_{34}H_{43}N_7O_9 \cdot 1\frac{3}{4}H_2O$ requires C, 56.30; H, 6.46; N, 13.52%]; ν_{max} (KBr) 3390, 3068, 2958, 2355, 2317, 1681, 1650, 1537, 1454, 1410, 1371, 1337, 1228, 1150, 1048, 1029, 980, 778, 749, 700, 662, 639 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.72 and 0.84 (6H, 2d, 1:1, *J*=6.8 Hz, *Me*$_2$CH), 1.20–1.26 (1H, m, Me$_2$C*H*), 1.75 (3H, s, CH$_3$), 2.37 (1H, dd, *J*=11.0, 13.4 Hz, 2-Ha), 2.89 (1H, dd, *J*=6.0, 13.4 Hz, 2-Hb), 3.61 (3H, s, OMe), 3.61–3.77 (3H, m, C*H*$_2$NH and 5-H), 3.81 (1H, br dd, *J*=6.2, 17.2 Hz, 1H of C*H*$_2$NH), 3.89 (1H, br dd, *J*=5.6, 17.2 Hz, 1H of C*H*$_2$NH), 3.96 (1H, br dd, *J*=5.8, 16.7 Hz, 1H of C*H*$_2$NH), 4.00–4.07 (1H, m, 1H of C*H*$_2$NH overlapped by the signal for 3-H), 4.04 (1H, br dd, *J*=6.0, 11.0 Hz, 3-H), 4.22 (1H, br dd, *J*=7.3, 9.3 Hz, 6-H), 4.32 (1H, dd, *J*=7.1, 17.8 Hz, 1H of C*H*$_2$NH), 4.42 (1H, m, *J*=8.3, 17.1 Hz, 1H of C*H*$_2$NH), 5.06–5.14 (2H, m, C*H*$_2$Ph), 6.03 (1H, t, *J*=5.0 Hz, N*H*CH$_2$), 7.28–7.40 (10H, m, 2× Ph), 7.51 (1H, t, *J*=6.0 Hz, N*H*CH$_2$), 7.82 (1H, d, *J*=7.3 Hz, N*H*CH), 7.99 (1H, t, *J*=5.2 Hz, N*H*CH$_2$), 8.68 (1H, dd, *J*=3.9, 7.4 Hz, N*H*CH$_2$); δ_C (126 MHz, $CDCl_3$) 15.8, 18.9, 23.1, 28.2, 41.0, 42.8, 43.5, 44.9, 52.3, 52.9, 57.2, 62.2, 67.4, 69.5, 71.7, 127.8, 128.2, 128.5, 128.7, 128.9, 128.9, 136.0, 136.9, 157.4, 160.9, 170.3, 170.8, 170.9, 171.1, 171.6; HRMS (ESI): MH$^+$, found 694.3191. $C_{34}H_{44}N_7O_9$ requires 694.3195.

*4.4.9. General procedure for the synthesis of non-racemic tripeptides **35**, **36**, **38**–**42**, **35′**, **36′** and **38′**–**42′**.* 4-Methylmorpholine (0.11 mL, 1 mmol) and EEDQ (247 mg, 1 mmol) were added to a stirred mixture of carboxylic acid (1 mmol), amine hydrochloride (1 mmol) and anhydrous dichloromethane (5 mL) and the mixture was stirred at room temperature for 24 h. (Addition of 4-methylmorpholine was omitted when free amine was used.) The volatile components were evaporated in vacuo. The residue was purified by FC. Fractions containing the product were combined and evaporated in vacuo to give a mixture of diastereomeric carboxamides, which were separated by MPLC. Fractions containing the products were evaporated in vacuo to give two diastereomeric non-racemic tripeptides.

The following compounds were prepared in this manner.

*4.4.9.1. Synthesis of tripeptides **35a** in **35a′**.* Prepared from the carboxylic acid **15a** (0.838 g, 1.55 mmol), methyl (*S*)-alaninate hydrochloride (**34**)(0.217 g, 1.55 mmol), 4-methylmorpholine (0.216 mL, 1.55 mmol) and EEDQ (0.915 g, 1.63 mmol); FC (dichloromethane/methanol, 30:1), MPLC (EtOAc/hexanes, 1:1).

*4.4.9.1.1. Methyl (S)-2-((1R,3R,5R,6R)-6-(benzyloxycarbonylamino)-7-oxo-3,5-diphenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)propanoate (**35a**).* Yield: 368 mg (43%) of white solid; mp 160–166 °C; [Found: C, 66.43; H, 6.07; N, 9.71. $C_{31}H_{32}N_4O_6$ requires C, 66.89; H, 5.79; N, 10.07%]; $[\alpha]_D^{24}$ −74.3 (*c* 0.146, EtOH); ν_{max} (KBr) 3316, 3064, 3036, 2950, 1737, 1715, 1687, 1681, 1548, 1541, 1454, 1437, 1243, 1217, 1156, 1058, 753, 698 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.51 (3H, d, *J*=7.2 Hz, 2′-Me), 2.70 (1H, br q, *J*=11.5 Hz, 2-Ha), 2.85–2.92 (1H, m, 2-Hb), 3.75 (3H, s, OMe), 3.92 (1H, dd, *J*=11.5, 5.3 Hz, 3-H), 4.28 (1H, d, *J*=11.7 Hz, 5-H), 4.53–4.61 (2H, m, 1-H and 3′-H), 4.68 (1H, br t, *J*=9.9 Hz, 6-H), 5.04 (2H, s, C*H*$_2$Ph), 5.48 (1H, d, *J*=8.3 Hz, NH), 6.97–7.13 (10H, m, 2× Ph), 7.26–7.34 (5H, m, Ph), 7.88 (1H, d, *J*=6.4 Hz, NH); δ_C (126 MHz, $CDCl_3$) 17.6, 42.5, 49.0, 52.6, 55.8, 64.7, 67.3, 68.6, 75.3, 127.8, 128.0, 128.0, 128.0, 128.0, 128.1, 128.1, 128.3, 128.6, 135.3, 135.8, 136.1, 156.0, 162.1, 168.4, 173.0; HRMS (ESI): MH$^+$, found 557.2400. $C_{31}H_{33}N_4O_6$ requires 557.2395.

*4.4.9.1.2. Methyl (S)-2-((1S,3S,5S,6S)-6-(benzyloxycarbonylamino)-7-oxo-3,5-diphenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)propanoate (**35a′**).* Yield: 420 mg (48%) of white solid; mp 70–74 °C; [Found: C, 66.68; H, 5.98; N, 9.84. $C_{31}H_{32}N_4O_6$ requires C, 66.89; H, 5.79; N, 10.07%]; $[\alpha]_D^{24}$ +62.7 (*c* 0.15, EtOH); ν_{max} (KBr) 3384, 3065, 3037, 1743, 1728, 1705, 1695, 1684, 1676, 1558, 1539, 1498, 1454, 1438, 1382, 1340, 1254, 1216, 1150, 1066, 1058, 760, 754, 697 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.51 (3H, d, *J*=7.4 Hz, 2′-Me), 2.70–2.92 (2H, m, 2-CH$_2$), 3.78 (3H, s, OMe), 3.99 (1H, dd, *J*=5.6, 11.0 Hz, 3-H), 4.42 (1H, d, *J*=11.3 Hz, 5-H), 4.50 (1H, dd, *J*=7.6, 11.3 Hz, 6-H), 4.58 (1H, br d, *J*=8.8 Hz, 1-H), 4.66 (1H, quintet, *J*=5.6 Hz, 3′-H), 5.07 (2H, s, C*H*$_2$Ph), 5.60 (1H, d, *J*=7.6 Hz, NH), 6.95–7.13 (10H, m, 2× Ph), 7.32 (5H, m, Ph), 8.04 (1H, d, *J*=7.1 Hz,

NH); δ_C (126 MHz, $CDCl_3$) 17.2, 43.4, 48.8, 52.5, 55.5, 65.6, 67.4, 68.3, 74.1, 127.9, 128.0, 128.0, 128.1, 128.1, 128.1, 128.3, 128.4, 128.7, 135.6, 135.7, 135.9, 156.2, 161.1, 168.6, 173.5; HRMS (ESI): MH^+, found 557.2401. $C_{31}H_{33}N_4O_6$ requires 557.2400.

*4.4.9.2. Synthesis of tripeptides **35c** in **35c′**.* Prepared from the carboxylic acid **15c** (143 mg, 0.33 mmol), methyl (*S*)-alaninate hydrochloride (**34**)(46 mg, 0.33 mmol), 4-methylmorpholine (0.036 mL, 0.33 mmol) and EEDQ (86 mg, 0.35 mmol); FC (EtOAc), MPLC (EtOAc/hexanes, 1:1).

*4.4.9.2.1. Methyl (S)-2-((1R,3R,5R,6R)-6-(benzyloxycarbonylamino)-5-isopropyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)propanoate (**35c**).* Yield: 55 mg (32%) of colourless oil; $[\alpha]_D^{24}$ −33.04 (*c* 0.115, $CDCl_3$); ν_{max} (liquid film) 3300, 3064, 3034, 2956, 2873, 1718, 1681, 1540, 1495, 1455, 1387, 1369, 1294, 1248, 1212, 1155, 1145, 1109, 1055, 977, 847, 735, 700 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.74 and 0.83 (6H, 2d, 1:1, *J*=6.9 Hz, *Me*$_2$CH), 1.23–1.37 (1H, m, C*H*Me$_2$), 1.47 (3H, d, *J*=7.3 Hz, 2′-Me), 2.63 (1H, ddd, *J*=9.3, 11.2, 13.0 Hz, 2-Ha), 2.76 (1H, ddd, *J*=0.9, 5.5, 13.0 Hz, 2-Hb), 3.31 (1H, dd, *J*=2.7, 11.7 Hz, 5-H), 3.73 (3H, s, OMe), 3.92 (1H, dd, *J*=5.5, 11.2 Hz, 3-H), 4.44 (1H, br d, *J*=9.3 Hz, 1-H), 4.59 (1H, br d, *J*=10.1 Hz, 6-H), 4.53 (1H, quintet, *J*=7.4 Hz, 3′-H), 5.08 and 5.13 (2H, 2d, 1:1, *J*=12.4 Hz, C*H*$_2$Ph), 5.91 (1H, d, *J*=9.1 Hz, NH), 7.29–7.40 (10H, m, 2× Ph), 7.84 (1H, d, *J*=6.9 Hz, NH); δ_C (126 MHz, $CDCl_3$), δ 16.3, 17.8, 18.9, 27.2, 43.6, 49.0, 52.7, 56.0, 56.9, 67.4, 68.8, 77.4, 127.7, 128.2, 128.4, 128.7, 128.8, 128.9, 136.3, 138.0, 156.0, 163.6, 168.4, 173.1; HRMS (ESI): MH^+, found 523.2553. $C_{28}H_{35}N_4O_6$ requires 523.2551.

*4.4.9.2.2. Methyl (S)-2-((1S,3S,5S,6S)-6-(benzyloxycarbonylamino)-5-isopropyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)propanoate (**35c′**).* Yield: 35 mg (20%) of colourless oil; $[\alpha]_D^{24}$ +82.86 (*c* 0.105, $CDCl_3$); ν_{max} (liquid film) 3334, 3063, 3034, 2958, 2873, 1739, 1716, 1696, 1681, 1538, 1496, 1455, 1388, 1369, 1292, 1256, 1212, 1173, 1144, 1057, 990, 848, 736, 701, 667, 606 cm^{-1}; δ_H (500 MHz, $CDCl_3$): δ_H 0.74 and 0.80 (6H, 2d, 1:1, *J*=6.9 Hz, *Me*$_2$CH), 1.26–1.29 (1H, m, *CH*Me$_2$), 1.45 (3H, d, *J*=7.4 Hz, 2′-Me), 2.66 (1H, ddd, *J*=9.5, 11.2, 12.9 Hz, 2-Ha), 2.80 (1H, ddd, *J*=0.9, 5.3, 12.9 Hz, 2-Hb), 3.45 (1H, dd, *J*=3.1, 11.2 Hz, 5-H), 3.90 (3H, s, COOMe), 3.92 (1H, dd, *J*=5.3, 11.2 Hz, 3-H), 4.47 (1H, br d, *J*=9.5 Hz, 1-H), 4.55 (1H, br d, *J*=8.6, 11.2 Hz, 6-H), 4.60 (1H, quintet, *J*=7.4 Hz, 2′-H), 5.07 and 5.10 (2H, 2d, 1:1, *J*=12.5 Hz, C*H*$_2$Ph), 5.99 (1H, d, *J*=8.4 Hz, NH), 7.28–7.40 (10H, m, 2× Ph), 8.08 (1H, d, *J*=7.3 Hz, NH); δ_C (126 MHz, $CDCl_3$) 16.2, 17.2, 19.1, 27.2, 44.1, 48.8, 52.6, 55.6, 57.3, 67.2, 68.3, 75.4, 127.7, 127.9, 128.3, 128.7, 128.7, 128.8, 136.2, 137.7, 156.3, 162.8, 168.8, 173.5; HRMS (ESI): MH^+, found 523.2548. $C_{28}H_{35}N_4O_6$ requires 523.2551.

*4.4.9.3. Synthesis of tripeptides **36c** in **36c′**.* Prepared from the carboxylic acid **18c** (904 mg, 2 mmol), methyl (*S*)-alaninate hydrochloride (**34**)(287 mg, 2 mmol), 4-methylmorpholine (0.220 mL, 2 mmol) and EEDQ (0.518 g, 2.1 mmol); FC (EtOAc), MPLC (EtOAc/hexanes, 1:4).

*4.4.9.3.1. Methyl (S)-2-((1R,3R,5R,6R)-6-(benzyloxycarbonylamino)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)propanoate (**36c**).* Yield: 320 mg (30%) of white solid; mp 186–188 °C; [Found: C, 64.70; H, 6.81; N, 10.36. $C_{29}H_{36}N_4O_6$ requires C, 64.91; H, 6.76; N, 10.44%]; $[\alpha]_D^{24}$ −107.5 (*c* 0.120, EtOH); $[\alpha]_D^{24}$ −107.4 (*c* 0.125, CH_2Cl_2); ν_{max} (KBr) 3354, 3067, 2971, 2956, 2872, 1756, 1716, 1681, 1654, 1573, 1514, 1459, 1430, 1387, 1347, 1337, 1306, 1292, 1258, 1215, 1188, 1153, 1130, 1080, 1053, 1025, 976, 965, 936, 917, 885, 849, 817, 779, 751, 701, 659 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.72 and 0.82 (6H, 2d, 1:1, *J*=7.0 Hz, *Me*$_2$CH), 1.26 (1H, td, *J*=7.0, 3.5 Hz, Me$_2$C*H*), 1.48 (3H, d, *J*=7.3 Hz, 2′-Me), 1.80 (3H, s, 1-Me), 2.25 (1H, dd, *J*=11.2, 13.0 Hz, 2-Ha), 3.23 (1H, dd, *J*=2.9, 11.6 Hz, 5-H), 3.31 (1H, dd, *J*=5.3, 13.0 Hz, 2-Hb), 3.70 (1H, dd, *J*=5.3, 11.2 Hz, 3-H), 3.74 (3H, s, OMe), 4.47 (1H, quintet, *J*=7.2 Hz, 2′-H), 4.59 (1H, dd, *J*=8.8, 11.6 Hz, 6-H), 5.09 and 5.12 (2H, 2d, *J*=12.4 Hz, C*H*$_2$Ph), 5.21 (1H, d, *J*=8.8 Hz, NH), 7.29–7.41 (10H, m, 2× Ph), 8.48 (1H, d, *J*=6.5 Hz, NH); δ_C (126 MHz, $CDCl_3$) 16.0, 17.3, 18.9, 23.4, 26.7, 49.0, 51.5, 52.3, 56.9, 64.3, 67.2, 68.9, 75.5, 127.5, 128.0, 128.2, 128.5, 128.5, 128.6, 136.0, 137.7, 155.8, 162.4, 171.2, 173.0; HRMS (ESI): MH^+, found 537.2707. $C_{29}H_{37}N_4O_6$ requires 537.2708.

*4.4.9.3.2. Methyl (S)-2-((1S,3S,5S,6S)-6-(benzyloxycarbonylamino)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)propanoate (**36c′**).* Yield: 181 mg (17%) of white solid; mp 159–162 °C; [Found: C, 64.75; H, 6.76; N, 10.44. $C_{29}H_{36}N_4O_6$ requires C, 64.91; H, 6.76; N, 10.44%]; $[\alpha]_D^{24}$ +81.7 (*c* 0.158, EtOH); $[\alpha]_D^{24}$ +85.4 (*c* 0.125, CH_2Cl_2); ν_{max} (KBr) 3624, 3583, 3301, 3065, 2958, 1748, 1711, 1693, 1650, 1547, 1430, 1375, 1334, 1277, 1205, 1155, 1109, 1084, 1064, 1026, 992, 961, 916, 900, 867, 848, 833, 783, 753, 720, 697, 664 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.73 and 0.82 (6H, 2d, 1:1, *J*=6.9 Hz, *Me*$_2$CH), 1.23–1.33 (1H, m, Me$_2$C*H*), 1.48 (3H, d, *J*=7.4 Hz, 2′-Me), 1.79 (3H, s, 1-Me), 2.27 (1H, dd, *J*=11.1, 13.0 Hz, 2-Ha), 3.21 (1H, dd, *J*=5.2, 13.0 Hz, 2-Hb), 3.31 (1H, dd, *J*=2.7, 11.4, Hz, 5-H), 3.75 (3H, s, OMe), 3.81 (1H, dd, *J*=5.2, 11.1 Hz, 3-H), 4.52–4.62 (2H, m, 6-H and 2′-H), 5.10 (2H, s, C*H*$_2$Ph), 5.52 (1H, d, *J*=8.6 Hz, NH), 7.27–7.42 (10H, m, 2× Ph), 8.39 (1H, d, *J*=7.2 Hz, NH); δ_C (126 MHz, $CDCl_3$) 16.0, 17.0, 19.0, 22.6, 26.9, 48.8, 52.2, 52.4, 57.2, 63.8, 67.1, 68.7, 75.2, 127.5, 127.9, 128.2, 128.4, 128.6, 128.6, 136.0, 137.7, 155.9, 162.1, 171.4, 173.7; HRMS (ESI): MH^+, found 537.2699. $C_{29}H_{37}N_4O_6$ requires 537.2708.

*4.4.9.4. Synthesis of tripeptides **38c** in **38c′**.* Prepared from the amine **20c** (88 mg, 0.25 mmol), (*S*)-Boc-alanine (**37**)(47 mg, 0.25 mmol) and EEDQ (65 mg, 0.26 mmol); FC (EtOAc), MPLC (EtOAc/hexanes, 1:4).

*4.4.9.4.1. tert-Butyl (1R,3R,5R,6R)-6-((S)-2-(tert-butoxycarbonylamino)propanamido)-5-isopropyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**38c**).* Yield: 58 mg (44%) of purple oil; $[\alpha]_D^{24}$ −61.6 (*c* 0.125, $CDCl_3$); ν_{max} (liquid film) 3310, 2976, 2935, 2878, 1738, 1704, 1698, 1684, 1558, 1538, 1520, 1505, 1496, 1456, 1446, 1392, 1368, 1244, 1226, 1159, 1112, 1070, 1041, 1023, 993, 853, 843, 734, 701, 666 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.68 and 0.85 (6H, 2d, 1:1, *J*=6.9 Hz, *Me*$_2$CH), 1.28–1.35 (1H, m, Me$_2$C*H*), 1.36 (3H, d, *J*=7.0 Hz, 2′-Me), 1.42 and 1.53 (18H, 2s, 1:1, 2× *t*-Bu), 2.49 (1H, ddd, *J*=0.7, 5.5, 13.2 Hz, 2-Ha), 2.66 (1H, ddd, *J*=9.3, 11.4, 13.2 Hz, 2-Hb), 3.13 (1H, dd, *J*=2.7, 11.7 Hz, 5-H), 3.89 (1H, dd, *J*=5.5, 11.4 Hz, 3-H), 4.11–4.18 (1H, m, 2′-H), 4.30 (1H, d, *J*=9.3 Hz, 1-H), 4.92 (1H, br s, NH), 5.17 (1H, dd, *J*=9.2, 11.7 Hz, 6-H), 6.51 (1H, br s, NH), 7.30–7.40 (5H, m, Ph); δ_C (126 MHz, $CDCl_3$) 16.3, 18.4, 27.5, 28.1, 28.2, 28.5, 44.1, 50.2, 53.9, 55.3, 69.1, 79.7, 80.3, 83.3, 127.6, 128.7, 128.9, 138.3, 155.5, 164.0, 169.1, 172.3; HRMS (ESI): MH^+, found 531.3171. $C_{28}H_{43}N_4O_6$ requires 531.3177.

*4.4.9.4.2. tert-Butyl (1S,3S,5S,6S)-6-((S)-2-((tert-butoxycarbonyl)amino)propanamido)-5-isopropyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**38c′**).* Yield: 38 mg (29%) of purple oil; $[\alpha]_D^{24}$ +62.6 (*c* 0.115, $CDCl_3$); ν_{max} (liquid film) 3321, 2978, 2930, 2873, 1739, 1713, 1695, 1683, 1495, 1455, 1392, 1368, 1246, 1227, 1155, 1110, 1068, 1050, 1027, 843, 741, 701 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.70 and 0.85 (6H, 2d, 1:1, *J*=6.9 Hz, *Me*$_2$CH), 1.36 (3H, d, *J*=7.1 Hz, 2′-Me), 1.30–1.36 (1H, m, Me$_2$C*H*), 1.45 and 1.53 (18H, 2s, 1:1, 2× *t*-Bu), 2.49 (1H, ddd, *J*=1.2, 5.6, 13.2 Hz, 2-Ha), 2.67 (1H, ddd, *J*=9.3, 11.4, 13.2 Hz, 2-Hb), 3.12 (1H, dd, *J*=2.8, 11.8 Hz, 5-H), 3.88 (1H, dd, *J*=5.6, 11.4 Hz, 3-H), 4.19–4.24 (1H, m, 2′-H), 4.31 (1H, d, *J*=9.3 Hz, 1-H), 5.05 (1H, d, *J*=5.1 Hz, NH), 5.18 (1H, dd, *J*=9.2, 11.8 Hz, 6-H), 6.59 (1H, d, *J*=9.2 Hz, NH), 7.30–7.40 (5H, m, Ph); δ_C (126 MHz, $CDCl_3$) 16.3, 18.5, 27.5, 28.1, 28.2, 28.5, 44.1, 50.4, 53.9, 55.2, 69.0, 79.7, 80.3, 83.3, 127.6, 128.7, 128.9, 138.2, 155.6, 164.2, 169.2, 172.5; HRMS (ESI): MH^+, found 531.3167. $C_{28}H_{43}N_4O_6$ requires 531.3177.

*4.4.9.5. Synthesis of tripeptides **39c** in **39c′**.* Prepared from the amine **22c** (373 mg, 1 mmol), (*S*)-Boc-alanine (**37**)(190 mg, 1 mmol) and EEDQ (247 mg, 1 mmol); FC (EtOAc), MPLC (EtOAc/hexanes, 1:4).

*4.4.9.5.1. tert-Butyl (1R,3R,5R,6R)-6-((S)-2-(tert-butoxycarbonylamino)propanamido)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**39c**).* Yield: 117 mg (21%) of pink crystals; mp 76–80 °C; $[\alpha]_D^{24}$ −66.2 (*c* 0.130, EtOH); $[\alpha]_D^{24}$ −58.8 (*c* 0.110, CH_2Cl_2); ν_{max} (KBr) 3388, 2978, 2925, 2873, 1693, 1681, 1504, 1455, 1390, 1368, 1293, 1251, 1219, 1165, 1088, 1070, 1028, 989, 941, 846, 753, 701, 651, 613 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.69 and 0.86 (6H, 2d, 1:1, *J*=6.9 Hz, Me_2CH), 1.29–1.35 (1H, m, Me_2CH), 1.36 (3H, d, *J*=7.1 Hz, 2′-Me), 1.45 and 1.54 (18H, 2s, 1:1, 2× *t*-Bu), 2.05 (3H, s, 1-Me), 2.31 (1H, dd, *J*=11.4, 13.2 Hz, 2-Ha), 2.67 (1H, dd, *J*=5.7, 13.2 Hz, 2-Hb), 3.09 (1H, dd, *J*=2.8, 11.7 Hz, 5-H), 3.92 (1H, dd, *J*=5.7, 11.4 Hz, 3-H), 4.17–4.26 (1H, m, 2′-H), 5.11 (1H, dd, *J*=9.2, 11.7 Hz, 6-H), 5.11 (1H, br s, NH), 6.62 (1H, br d, *J*=9.2 Hz, NH), 7.26–7.36 (3H, m, *o*,*p*-Ph), 7.36–7.41 (2H, m, *m*-Ph); δ_C (126 MHz, $CDCl_3$) 14.4, 16.3, 18.5, 21.3, 22.5, 27.5, 28.2, 28.5, 52.9, 53.8, 60.6, 62.7, 70.0, 79.3, 83.0, 127.5, 128.6, 128.8, 138.5, 163.3, 170.6, 171.4, 172.5; HRMS (ESI): MH^+, found 545.3335. $C_{29}H_{45}N_4O_6$ requires 545.3334.

*4.4.9.5.2. tert-Butyl (1S,3S,5S,6S)-6-((S)-2-(tert-butoxycarbonylamino)propanamido)-5-isopropyl-1-methyl-7-oxo-3-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**39c′**).* Yield: 150 mg (28%) of pink crystals; mp 167–169 °C; $[\alpha]_D^{24}$ +52.7 (*c* 0.112, EtOH); $[\alpha]_D^{24}$ +35.0 (*c* 0.100, CH_2Cl_2); ν_{max} (KBr) 3418, 2974, 1681, 1651, 1514, 1454, 1369, 1288, 1251, 1162, 1084, 1048, 880, 840, 794, 746, 700, 667 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.68 and 0.86 (6H, 2d, 1:1, *J*=6.9 Hz, Me_2CH), 1.28–1.33 (1H, m, Me_2CH), 1.36 (3H, d, *J*=7.0 Hz, 2′-Me), 1.42 and 1.55 (18H, 2s, 2× *t*-Bu), 2.05 (3H, s, 1-Me), 2.30 (1H, dd, *J*=11.3, 13.2 Hz, 2-Ha), 2.67 (1H, dd, *J*=5.7, 13.2 Hz, 2-Hb), 3.09 (1H, dd, *J*=2.6, 11.8 Hz, 5-H), 3.92 (1H, dd, *J*=5.7, 11.3 Hz, 3-H), 4.12–4.20 (1H, m, 2′-H), 4.94 (1H, br s, NH), 5.09 (1H, dd, *J*=9.5, 11.8 Hz, 6-H), 6.55 (1H, d, *J*=9.5 Hz, NH), 7.27–7.36 (3H, m, *o*,*p*-Ph), 7.37–7.41 (2H, m, *m*-Ph); δ_C (126 MHz, $CDCl_3$) 11.0, 16.1, 17.7, 18.3, 22.3, 23.0, 28.0, 28.3, 30.4, 52.8, 53.6, 60.4, 62.4, 70.0, 79.1, 82.8, 127.4, 128.4, 128.6, 138.4, 162.9, 170.3, 172.1; HRMS (ESI): MH^+, found 545.3330. $C_{29}H_{45}N_4O_6$ requires 545.3334.

*4.4.9.6. Synthesis of tripeptides **40b** in **40b′**.* Prepared from the carboxylic acid **16b** (1.08 g, 2 mmol), methyl (*S*)-alaninate hydrochloride (**34**) (287 mg, 2 mmol), 4-methylmorpholine (0.220 mL, 2 mmol) and EEDQ (0.518 g, 2.1 mmol); FC (EtOAc), MPLC (EtOAc/hexanes, 2:1).

*4.4.9.6.1. Methyl (S)-2-((1S,3S,5R,6R)-6-(benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-7-oxo-5-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)propanoate (**40b**).* Yield: 442 mg (35%) of pale yellowish solid; mp 86–90 °C; [Found: C, 59.32; H, 4.99; N, 8.78. $C_{31}H_{30}Cl_2N_4O_6$ requires C, 59.53; H, 4.83; N, 8.96%]; $[\alpha]_D^{24}$ +6.9 (*c* 0.124, EtOH); ν_{max} (KBr) 3314, 3065, 3036, 2953, 1725, 1710, 1686, 1578, 1560, 1536, 1456, 1440, 1380, 1344, 1290, 1246, 1213, 1152, 1057, 783, 753, 698 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.49 (3H, d, *J*=7.2 Hz, 2′-Me), 2.98 (1H, ddd, *J*=4.1, 10.4, 14.1 Hz, 2-Ha), 3.48 (1H, ddd, *J*=4.3, 10.2, 14.1 Hz, 2-Hb), 3.76 (1H, d, *J*=11.7 Hz, 5-H), 3.77 (3H, s, OMe), 4.61 (1H, quintet, *J*=7.0 Hz, 2′-H), 4.92 (1H, br t, *J*=10.1 Hz, 6-H), 4.99 (2H, s, CH_2Ph), 5.01 (1H, dd, *J*=4.3, 10.4 Hz, 3-H), 5.30 (1H, d, *J*=9.2 Hz, NH), 5.49 (1H, dd, *J*=4.1, 10.2 Hz, 1-H), 7.00–7.40 (13H, m, 2× Ph and C_6H_3), 7.62 (1H, d, *J*=5.4 Hz, NH); δ_C (75.5 MHz, $CDCl_3$) 18.2, 33.6, 49.0, 52.7, 55.3, 58.1, 63.8, 67.4, 71.1, 127.8, 128.4, 128.7, 128.7, 128.7, 128.9, 129.5, 130.2, 130.5, 132.4, 134.8, 138.9, 155.7, 161.1, 168.5, 173.0; HRMS (ESI): MH^+, found 625.1620. $C_{31}H_{31}Cl_2N_4O_6$ requires 625.1621.

*4.4.9.6.2. Methyl (S)-2-((1R,3R,5S,6S)-6-(benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-7-oxo-5-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)propanoate (**40b′**).* Yield: 336 mg (27%) of pale yellowish solid; mp 72–78 °C; [Found: C, 59.41; H, 4.90; N, 8.78. $C_{31}H_{30}Cl_2N_4O_6$ requires C, 59.53; H, 4.83; N, 8.96%]; $[\alpha]_D^{24}$ −18.2 (*c* 0.208, EtOH); ν_{max} (KBr) 3328, 3065, 3036, 2953, 1724, 1690, 1578, 1543, 1533, 1499, 1455, 1441, 1381, 1335, 1247, 1213, 1154, 1064, 1051, 912, 778, 766, 698 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.49 (3H, d, *J*=7.2 Hz, 2′-Me), 3.05 (1H, ddd, *J*=4.2, 10.2, 13.9 Hz, 2-Ha), 3.43 (1H, ddd, *J*=4.0, 10.2, 13.9 Hz, 2-Hb), 3.76 (1H, d, *J*=12.0 Hz, 5-H), 3.79 (3H, s, OMe), 4.61 (1H, quintet, *J*=7.3 Hz, 2′-H), 4.93 (1H, br t, *J*=10.4 Hz, 6-H), 4.98 (1H, dd, *J*=4.0, 10.2 Hz, 3-H), 4.99 (2H, s, CH_2Ph), 5.26 (1H, d, *J*=9.2 Hz, NH), 5.50 (1H, dd, *J*=4.2, 10.2 Hz, 1-H), 7.41–6.98 (13H, m, 2× Ph and C_6H_3), 7.53 (1H, d, *J*=7.4 Hz, 2′-NH); δ_C (126 MHz, $CDCl_3$) 18.2, 33.4, 48.8, 52.7, 55.1, 58.2, 63.9, 67.4, 70.6, 127.7, 128.3, 128.6, 128.6, 128.8, 129.4, 130.2, 130.4, 132.3, 134.7, 135.7, 138.9, 155.6, 161.2, 168.6, 172.8; HRMS (ESI): MH^+, found 625.1599. $C_{31}H_{31}Cl_2N_4O_6$ requires 625.1621.

*4.4.9.7. Synthesis of tripeptides **41d** in **41d′**.* Prepared from the carboxylic acid **19d** (260 mg, 0.5 mmol), methyl (*S*)-alaninate hydrochloride (**34**) (63 mg, 0.5 mmol), 4-methylmorpholine (0.055 mL, 0.5 mmol) and EEDQ (0.113 g, 0.5 mmol); FC (EtOAc), MPLC (EtOAc/hexanes, 1:6).

*4.4.9.7.1. Methyl (S)-2-((1R,3S,5R,6R)-6-(benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-7-oxo-5-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)propanoate (**41d**).* Yield: 46 mg (15%) of white solid; mp 48–49 °C; $[\alpha]_D^{24}$ −128.2 (*c* 0.104, EtOH); ν_{max} (KBr) 3308, 3065, 3033, 2957, 2935, 2875, 1716, 1673, 1580, 1537, 1532, 1454, 1436, 1393, 1375, 1252, 1204, 1187, 1150, 1117, 1083, 1041, 984, 950, 939, 848, 779, 750, 735, 697, 652, 635, 607 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.82 and 1.08 (6H, 2d, 1:1, *J*=6.8 Hz, Me_2CH), 1.44 (3H, d, *J*=7.2 Hz, 2′-Me), 1.83 (3H, s, 1-Me), 1.85–1.92 (1H, m, Me_2CH), 2.63 (1H, dd, *J*=8.6, 13.7 Hz, 2-Ha), 3.01 (1H, dd, *J*=3.4, 6.5 Hz, 5-H), 3.31 (1H, dd, *J*=7.7, 13.7 Hz, 2-Hb), 3.71 (3H, s, OMe), 4.54 (1H, quintet, *J*=7.4 Hz, 2′-H), 4.76 (1H, br t, *J*=7.9 Hz, 6-H), 5.12 (2H, s, CH_2Ph), 5.36 (1H, t, *J*=8.2 Hz, 3-H), 5.53 (1H, br d, *J*=9.2 Hz, NH), 7.20 (1H, t, *J*=8.0 Hz, *p*-C_6H_3), 7.29–7.40 (7H, m, Ph and *m*-C_6H_3), 9.02 (1H, d, *J*=7.0 Hz, NH); δ_C (75.5 MHz, $CDCl_3$) 17.2, 17.9, 19.6, 23.8, 27.5, 45.4, 48.6, 52.6, 55.3, 58.1, 64.3, 67.4, 67.6, 128.3, 128.4, 128.6, 129.3, 130.0, 134.9, 136.1, 136.8, 155.9, 162.3, 171.5, 173.5; HRMS (ESI): MH^-, found 603.1783. $C_{29}H_{33}Cl_2N_4O_6$ requires 603.1788.

*4.4.9.7.2. Methyl (S)-2-((1S,3R,5S,6S)-6-(benzyloxycarbonylamino)-3-(2,6-dichlorophenyl)-7-oxo-5-phenylhexahydropyrazolo[1,2-a]pyrazole-1-carboxamido)propanoate (**41d′**).* Yield: 49 mg (16%) of white solid; mp 49–50 °C; $[\alpha]_D^{24}$ +105.7 (*c* 0.106, EtOH); ν_{max} (KBr) 3305, 3066, 3034, 2957, 2934, 2877, 1715, 1673, 1580, 1531, 1454, 1436, 1393, 1374, 1250, 1205, 1152, 1116, 1082, 1041, 983, 951, 912, 862, 846, 779, 751, 736, 699, 668, 632 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.80 and 1.08 (6H, 2d, 1:1, *J*=6.8 Hz, Me_2CH), 1.47 (3H, d, *J*=7.3 Hz, 2′-Me), 1.83 (3H, s, 1-Me), 1.85–1.93 (1H, m, Me_2CH), 2.62 (1H, dd, *J*=8.9, 13.8 Hz, 2-Ha), 3.00 (1H, dd, *J*=3.0, 6.6 Hz, 5-H), 3.50 (1H, dd, *J*=7.5, 13.8 Hz, 2-Hb), 3.70 (3H, s, OMe), 4.55 (1H, quintet, *J*=7.3 Hz, 2′-H), 4.73 (1H, br t, *J*=7.8 Hz, 6-H), 5.11 (2H, s, CH_2Ph), 5.38 (1H, t, *J*=8.2 Hz, 3-H), 5.53 (1H, br d, *J*=8.4 Hz, NH), 7.18 (1H, t, *J*=8.0 Hz, *p*-C_6H_3), 7.28–7.38 (7H, m, Ph and *m*-C_6H_3), 9.27 (1H, d, *J*=7.1 Hz, NH); δ_C (75.5 MHz, $CDCl_3$) 17.0, 17.6, 19.7, 24.5, 27.4, 44.8, 48.6, 52.5, 54.9, 58.3, 64.8, 67.3, 67.6, 128.3, 128.6, 129.1, 129.9, 131.0, 135.5, 136.2, 136.6, 155.9, 162.2, 171.8, 173.4; HRMS (ESI): MH^+, found 605.1930. $C_{29}H_{35}Cl_2N_4O_6$ requires 605.1928.

*4.4.9.8. Synthesis of tripeptides **42d** in **42d′**.* Prepared from the amine **23d** (856 mg, 1.9 mmol), Boc-(*S*)-alanine (**37**) (360 mg, 2 mmol), 4-methylmorpholine (0.22 mL, 2 mmol) and EEDQ (469 mg, 2 mmol); FC (EtOAc), MPLC (EtOAc/hexanes, 1:4).

*4.4.9.8.1. tert-Butyl (1R,3S,5R,6R)-6-((S)-2-(tert-butoxycarbonylamino)-propanamido)-3-(2,6-dichlorophenyl)-5-isopropyl-1-methyl-7-oxohexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**42d**).* Yield: 115 mg (10%) of white solid; mp 55–57 °C; $[\alpha]_D^{24}$ −107.5 (*c* 0.120, EtOH); ν_{max} (KBr) 3455, 2975, 2930, 2878, 1699, 1581, 1561, 1520, 1452, 1438, 1392, 1368, 1290, 1251, 1162, 1085, 1070, 1046, 884, 783, 763, 668, 656, 622, 605 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.88 and 1.08 (6H, 2d, 1:1, *J*=6.8 Hz, Me_2CH), 1.39 (3H, d, *J*=7.3 Hz, 2′-Me), 1.42 and 1.52 (18H, 2s, 2× *t*-Bu), 1.83 (3H, s, 1-Me), 1.95–2.04 (1H,

m, Me_2CH), 2.49 (1H, dd, J=8.0, 13.0 Hz, 2-Ha), 2.99 (1H, br t, J=3.0 Hz, 5-H), 3.14 (1H, dd, J=9.5, 13.0 Hz, 2-Hb), 4.15–4.25 (1H, m, 2′-H), 4.83 (1H, dd, J=3.0, 8.8 Hz, 6-H), 5.09 (1H, br d, J=6.7 Hz, NH), 5.18 (1H, dd, J=8.0, 9.5 Hz, 3-H), 6.80 (1H, br d, J=7.8 Hz, NH), 7.18 (1H, t, J=8.0 Hz, p-C_6H_3), 7.30 and 7.33 (2H, 2d, 1:1, J=8.0 Hz, m-C_6H_3); δ_C (75.5 MHz, $CDCl_3$) 18.0, 18.9, 20.3, 22.4, 27.4, 28.1, 28.4, 45.8, 50.2, 55.1, 57.4, 66.8, 77.2, 79.9, 82.8, 129.0, 129.8, 131.3, 136.2, 155.4, 162.9, 170.3, 172.3; HRMS (ESI): $[M-H]^-$, found 613.2610. $C_{29}H_{41}Cl_2N_4O_6$ requires 613.2554.

*4.4.9.8.2. tert-Butyl (1S,3R,5S,6S)-6-((S)-2-(tert-butoxycarbonylamino)-propanamido)-3-(2,6-dichlorophenyl)-5-isopropyl-1-methyl-7-oxohexahydropyrazolo[1,2-a]pyrazole-1-carboxylate (**42d′**).* Yield: 195 mg (17%) of white solid; mp 76–80 °C; $[\alpha]_D^{24}$ +72.7 (c 0.172, EtOH); ν_{max} (KBr) 3430, 2978, 2932, 1707, 1579, 1561, 1507, 1452, 1438, 1393, 1367, 1289, 1249, 1163, 1129, 1087, 1065, 1023, 929, 845, 784, 763, 721, 688, 661, 639 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.89 and 1.09 (6H, 2d, 1:1, J=6.8 Hz, Me_2CH), 1.39 (3H, d, J=7.3 Hz, 2′-Me), 1.45 and 1.52 (18H, 2s, 2× t-Bu), 1.83 (3H, s, 1-Me), 1.95–2.05 (1H, m, Me_2CH), 2.50 (1H, dd, J=8.0, 12.9 Hz, 2-Ha), 2.98 (1H, br t, J=2.5 Hz, 5-H), 3.14 (1H, dd, J=9.5, 12.9 Hz, 2-Hb), 4.24–4.34 (1H, m, 2′-H), 4.81 (1H, dd, J=2.5, 8.7 Hz, 6-H), 5.12 (1H, br d, J=6.2 Hz, NH), 5.18 (1H, dd, J=8.0, 9.5 Hz, 3-H), 6.97 (1H, d, J=8.7 Hz, NH), 7.18 (1H, t, J=8.0 Hz, p-C_6H_3), 7.29 and 7.34 (2H, 2d, 1:1, J=8.0 Hz, m-C_6H_3); δ_C (75.5 MHz, $CDCl_3$) 18.1, 19.1, 20.3, 22.3, 28.1, 28.5, 45.9, 50.4, 55.0, 57.4, 66.7, 77.2, 80.0, 82.7, 129.0, 129.9, 131.3, 134.2, 136.2, 155.4, 163.1, 170.2, 172.3; HRMS (ESI): MH^-, found 611.2426. $C_{29}H_{41}Cl_2N_4O_6$ requires 611.2403.

*4.4.10. X-ray structure analysis for compounds **5c**, **5d**, **9d**, **11b**, **11d**, **21b**, **36c′**, **39c′** and **40b′**.* For X-ray structure determination, the crystals of the aforementioned compounds were mounted on the tip of glass fibres and transferred to the goniometer head. Data were collected on a Nonius Kappa CCD diffractometer using monochromated Mo Kα radiation at room temperature by using Nonius Collect software.[20] Data reduction and integration were performed with the software package DENZO-SMN.[21] The coordinates of all of the non-hydrogen atoms were found via direct methods using the SIR97 or SHELXS-97 structure solution programs.[22,23] A full-matrix least-squares refinement on F^2 magnitudes with anisotropic displacement parameters for all non-hydrogen atoms using SHELXL-97 was employed.[23] All hydrogen atoms were initially located in difference Fourier maps. All H atoms attached to carbon were subsequently treated as riding atoms in geometrically idealized positions with bond lengths C–H of 0.96 Å for methyl, 0.97 Å for methylene, 0.98 Å for methine and 0.93 Å for aromatic C–H bonds. The corresponding displacement parameters U_{iso}(H) were 1.5-times higher than those of the carrier methyl carbons and 1.2-times higher than all other hydrogen bearing carbon atoms.

Hydrogen atoms attached to nitrogens and (possibly) taking part in hydrogen bonding were found in the difference electron density maps and refined isotropically with the constraint U_{iso}(H)= 1.2 U_{eq}(N). If the refinement yielded unreasonable hydrogen positions, their coordinates were not refined at all.

Figures depicting the structures were prepared by ORTEP3.[24]

CCDC 901504–901512 contain the supplementary crystallographic data for structures **5c**, **5d**, **9d**, **11b**, **11d**, **21b**, **36c′**, **39c′** and **40b′**, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.4.11. Determination of inhibitory activities on MurD and Ddl. The inhibition of MurD and DdlB was determined using the Malachite green assay, as described previously,[19] with slight modifications. A mixture with a final volume of 50 μL was used, containing:

MurD: 50 mM Hepes, pH 8.0, 5 mM $MgCl_2$, 0.005% Triton X-114, 80 μM UDP-MurNAc-L-Ala, 100 μM D-Glu, 400 μM ATP, purified MurD from *Escherichia coli*[25] (diluted in 50 mM Hepes, and 1 mM dithiothreitol), and 250 μM of the compound dissolved in DMSO.

DdlB: 50 mM Hepes, pH 8.0, 5 mM $MgCl_2$, 6.5 mM $(NH_4)_2SO_4$, 10 mM KCl, 0.005% Triton X-114, 700 μM D-Ala, 500 μM ATP, purified DdlB from *E. coli* (diluted in 50 mM Hepes, and 1 mM dithiothreitol), and 250 μM of compound dissolved in DMSO.

The final concentration of DMSO was 5% (v/v). The reaction mixture was incubated at 37 °C for 15 (MurD) and 20 min (DdlB), respectively, then quenched with 100 μL of Biomol reagent. Absorbance at 650 nm was read after 5 min. Residual activities were calculated with respect to control assay without the compound and with DMSO.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2013.05.122. These data include MOL files and InChiKeys of the most important compounds described in this article.

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