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Short and Novel Stereospecific Synthesis of Trisubstituted 2,5-Diketopiperazines

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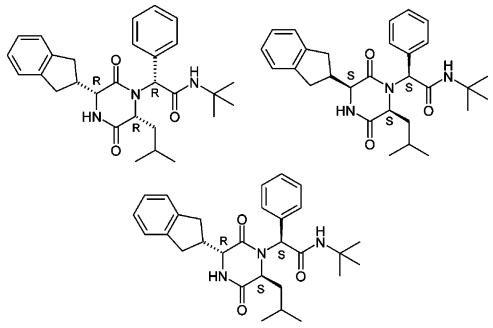


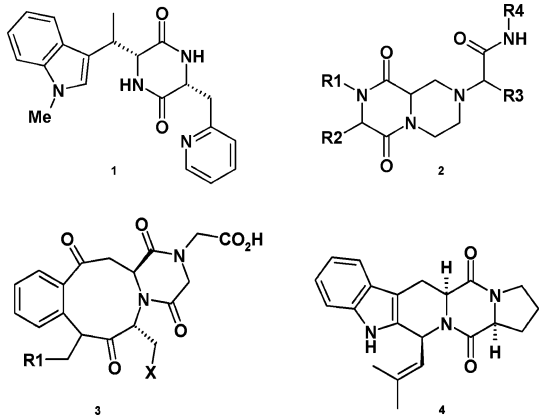
Novel syntheses of chiral trisubstituted 2,5-diketopiperazines using multicomponent Ugi reactions were developed.

Introduction

2,5-Diketopiperazines (DKPs) are an interesting class of heterocycles, as they show varied pharmacological activity (Chart 1). For example, compound **1** is reported to be an inhibitor of a platelet-activating factor.[1] The conformationally restricted analogue **2** has been studied as β-turn mimetics.[2] Tricylic analogues of 2,5-diketopiperazine **3** are CCK_2 receptor antagonists.[3] The 2,5-diketopiperazine motif has been found in the structures of alkaloid natural products such as demethoxyfumitremorgin C **4**.[4]

We are concerned with the design and synthesis of these topologically attractive and functionally diverse scaffolds in the context of a selective orally bioavailable oxytocin antagonist. We report here the facile stereoselective synthesis of enantiometrically pure trisubstituted 2,5-diketopiperazines with three chiral centers represented by the general structures **5a, 5b**, and **5c** (Chart 2).

CHART 1



Results and Discussion

The trisubstituted 2,5-diketopiperazines are assembled by the cyclization of dipeptide esters via intramolecular amide bond formation.[5] The intermediate dipeptide esters **11** are readily obtained utilizing a four component Ugi coupling reaction (Scheme 1), previously carried out on the solid phase.[6] The hydrochloride of *R*-leucine methyl

(1) Shimazaki, N.; Hemmi, K.; Nakaguti, O.; Miyazaki, Y.; Hashimoto, M. European Patent Application EP 243,122; Oct., 28, 1987, p 53.

(2) Golebiowski, A.; Klopfenstein, S. R.; Chen, J. J.; Shao, X. *Tetrahedron Lett.* **2000**, *41*, 4841−4844.

(3) McDonald, I. M.; Dunstone, D. J.; Kalindjian, S. B.; Linney, I. D.; Low, C. M. R.; Pether, M. J.; Steel, K. I. M.; Tozer, M. J.; Vinter, J. G. *J. Med. Chem.* **2000**, *43*, 3518−3529.

(4) Wang, H.; Ganesan, A. *Org. Lett.* **1999**, *1*, 1647−1649.

(5) Fischer, P. M. *J. Peptide Sci.* **2003**, *9*, 9−35.

(6) Szardenings, A. K.; Burkoth, T. S.; Lu, H. H.; Tien, D. W.; Campbell, D. A. *Tetrahedron* **1997**, *53*, 6573−6593.

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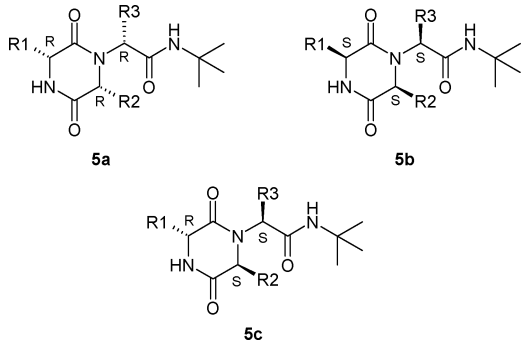
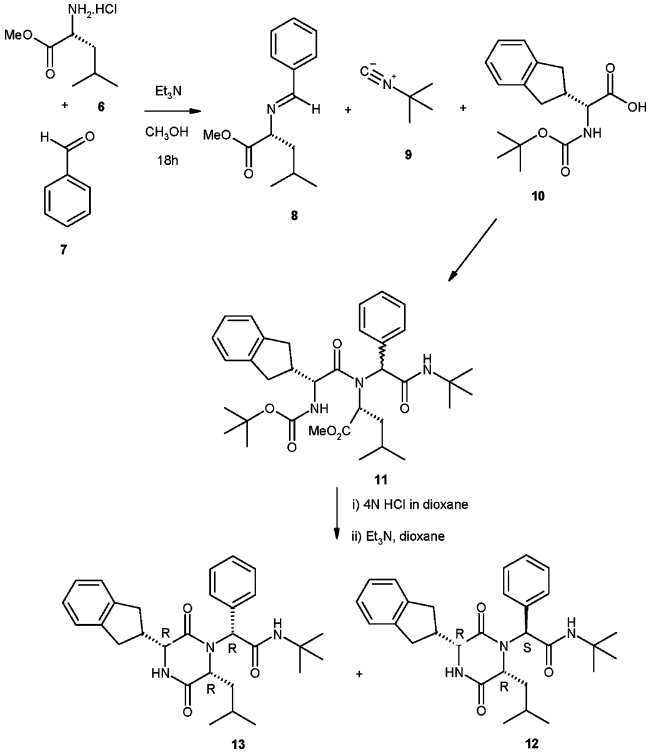
CHART 2

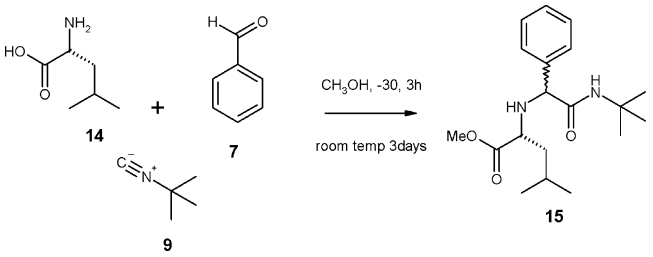


SCHEME 1



ester **6** and benzaldehyde **7** were mixed together in methanol with an equivalent of triethylamine to form the imine **8**, which was not isolated. To this solution was added *t*-butylisonitrile **9** and the *R*-indanyl glycine **10**. The crude intermediate dipeptide ester **11** was deprotected with 4 N hydrogen chloride in dioxane for 4 h. This not only removed the *t*-Boc group of the glycine but also gave some spontaneous cyclization to the 2,5-diketopiperazine. The cyclization was driven to completion by the addition of triethylamine. This gave two trisubstituted 2,5-diketopiperazines in a ratio of 3:1. Proton NMR experiments showed a medium to strong nOe from the CH of the indanyl to one of the hydrogens of the CH_2 of the isobutyl group, indicating that the indanyl moiety and the isobutyl group are cis orientated to each other across the diketopiperazine as expected. The two isomers were the RRS trisubstituted 2,5-diketopiperazine **12** and the RRR trisubstituted 2,5-diketopiperazine **13**, but it was not known which was the major product. The minor product **13** was found to have a greater potency as an oxytocin antagonist,[7] and X-ray crystallography[8] showed it to be the RRR trisubstituted 2,5-diketopiperazine **13**.

SCHEME 2



The partial stereocontrol comes from the addition of the isonitrile **9** to the less hindered face of the imine **8**. Attempts to alter this ratio in favor of the RRR isomer **13** by changing the temperature, solvent, or time course of the Ugi reaction were unsuccessful.

Several alternative syntheses of DKPs[9] were considered to selectively prepare the required RRR isomer **13**. However, none were efficient or sufficiently selective. It was envisaged that fixing the stereochemistry of the exocyclic center prior to the cyclization to form the DKP would be a potential advantage in our efforts to prepare the RRR isomer **13**. The most attractive of the possibilities was to synthesize the secondary amine **15** and then to acylate using *R-Boc*-indanyl glycine **10**. A survey of the literature found an efficient synthesis of analogues of the secondary amine **15**[10] via a four center, three-component Ugi reaction; however, the stereochemistry of the major diastereoisomer was not reported. Thus, the three components, *R*-leucine **14**, benzaldehyde **7**, and *t*-butylisonitrile **9** (Scheme 2), were dissolved/suspended in methanol at −30 °C for 3 h, and then the reaction mixture was stirred at room temperature for 3 days to produce predominately one isomer (8:1 mixture of diastereoisomers).

Unfortunately, the amino ester **15** failed to undergo intramolecular acylation with *R-Boc*-indanyl glycine **10**, probably due to steric bulk of both components. Furthermore, formylation using formyl acetic anhydride took overnight to produce **16** in a 70% yield (Scheme 3). Formylation was also attempted on the corresponding acid **17**. Hydrolysis of the ester **15** to the amino acid **17** was achieved using lithium hydroxide in aqueous methanol. Formylation of the amine **17** using formyl acetic anhydride occurred very rapidly (2 h at room temp) in quantitative yield to give **19**. One possibility of the difference in rates of the reactions could be explained by the formation of the mixed anhydride **18**, followed by an intramolecular acylation of the amine. This suggested that making the corresponding mixed anhydride with *R*-indanyl glycine **10** would enable the preparation of the required intermediate dipeptide ester **21**. Methylation of acid **19** gave ester **16**, which showed that the stereochemistry of both centers was conserved.

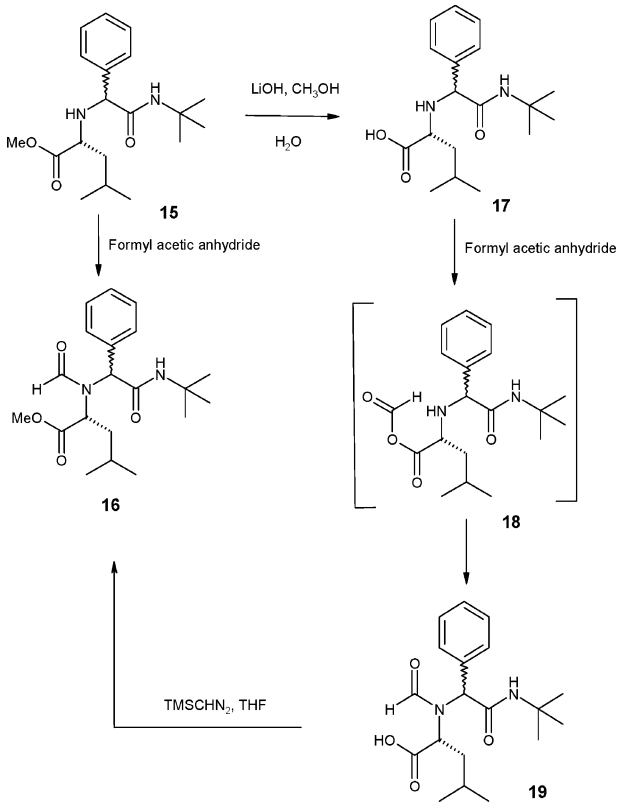
R-Boc-indanyl glycine **10** was treated with isopropyl chloroformate in the presence of *N*-methylmorpholine at −20 °C to form the mixed anhydride **20** (Scheme 4). To

(7) Borthwick, A. D.; Hatley, R. J.; Hickey, D. M. B.; Liddle, J.; Livermore, D. G. H.; Mason, A. M.; Miller, N. D.; Nerozzi, F.; Sollis, S. L.; Szardenings, A. K.; Wyatt, P. G. PCT Int. Appl. 2003, p 90; CODEN: WO 2003053443 A1 20030703.

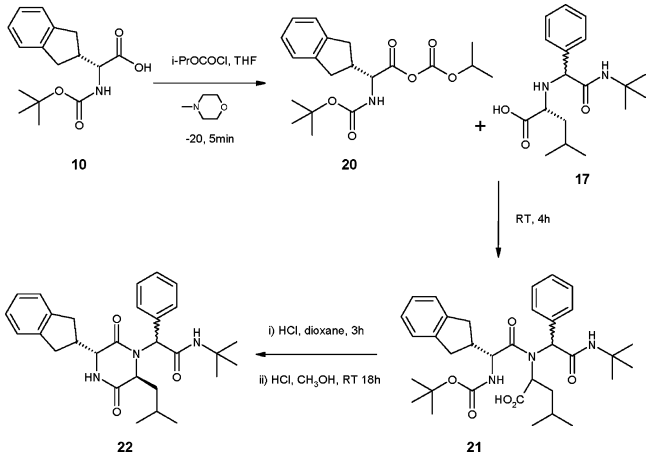
(8) X-ray to be published later.

(9) Dinsmore, C. J.; Beshore, D. C. *Tetrehedron* **2002**, *58*, 3297−3312.

SCHEME 3



SCHEME 4



this was added amino acid **17**, and the reaction was warmed to room temperature to give **21**. The acyclic intermediate **21** was taken crude through to the final DKP **22.** The major isomer **22** isolated was obtained in 45% overall yield from *R-Boc*-indanyl glycine. Surprisingly, the stereochemistry of the indanyl and isobutyl substituents in **22** was shown to be trans by rotational nuclear Overhauser effect spectroscopy (ROSEY) NMR.

It was reasoned that the inversion of stereochemistry in the formation of **22** had occurred during the preparation of its precursor **21**. To investigate this, *R-Boc*-indanyl glycine **10** was replaced with the *S-Boc*-indanyl glycine **23**, and the reaction was repeated, keeping the other components the same. The major isomer isolated was the SSS isomer **24** (Scheme 5). The structure of **24** was confirmed by ^{1}H and ^{13}C NMR spectroscopy, which were

SCHEME 5

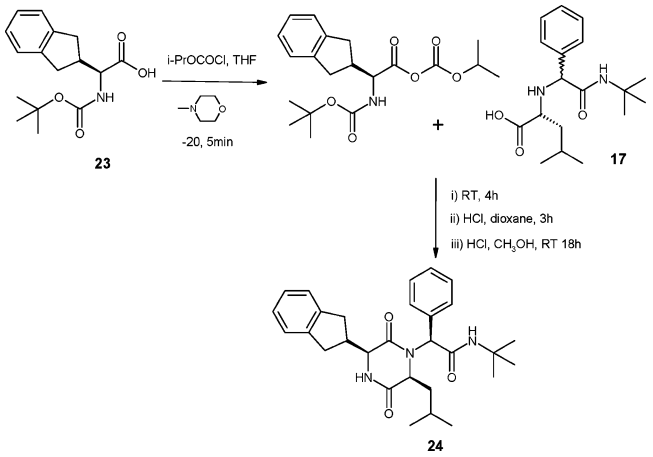
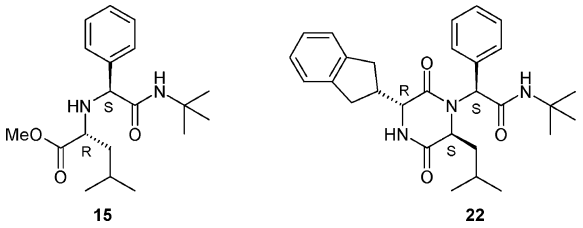


CHART 3



identical to that of the RRR isomer **13**, but the retention times by chiral HPLC were different. In addition, the circular dichroism spectra of DKP **13** and DKP **24** were mirror images.

Hence, the leucyl amide is inverted in the preparation of intermediate dipeptide ester **21**, and because the isomer **24** is chirally pure with the SSS configuration, this means that the stereochemistry of the phenyl glycine moiety is also S and is assumed to be retained in this reaction. Also, the chirality of the indanyl glycine is retained during the reaction.

Therefore, in the original secondary amine derivative **15,** the chirality of the phenyl glycine moiety is S, and the major isomer is RS. Also, the chirality of DKP **22** is RSS (Chart 3).

Further investigation of the reaction of the anhydride **20** of the *R-Boc*-indanyl glycine **10** with the amine **17** showed that if the reaction temperature of the acylation is kept at −20 °C, then two major isomers were isolated, the cis isomer **12** and the trans isomer **22** (Scheme 6).

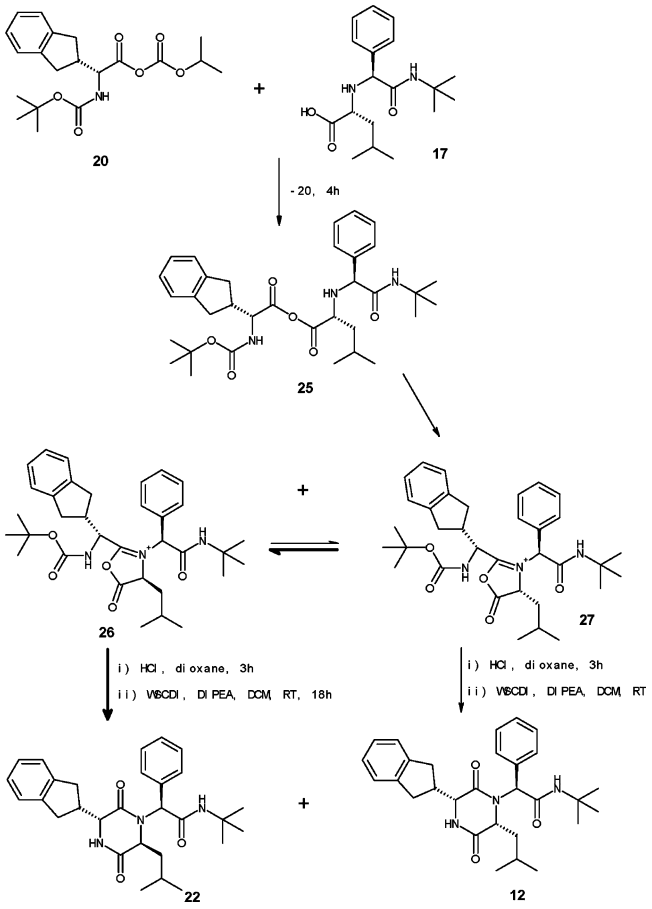
The epimerization of the leucyl group could occur at the mixed anhydride **25** stage or at a possible cyclic intermediate. Molecular modeling[11] indicates that the possible intermediate **26** is of lower energy than intermediate **27** by 40 kJ/mol. It is predicted, at room temperature, that the intermediate **27** racemizes at the leucine center to the more favored intermediate **26**, driving the reaction to form DKP **22**. In contrast, at −20 °C, racemization of **27** is reduced, and a mixture of DKP **22** and DKP **12** is formed (2:1).

It has been shown that using *R*-leucine and *S-Boc*-indanyl glycine, the SSS DKP **24** can be synthesized.

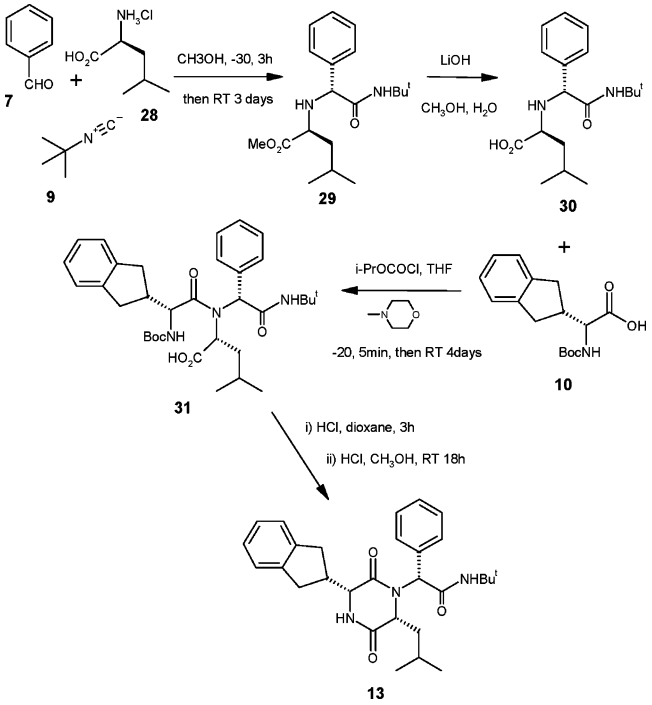
(10) Ugi, I.; Hörl, W.; Hanusch-Kompa, C.; Schmid, T.; Herdtweck, E. *Heterocycles* **1998**, *47*, 965−975.

(11) Both isomers were investigated using Macromodel; full details are in the Supporting Information.

SCHEME 6



SCHEME 7



Therefore, by starting with *S*-leucine and *R-Boc*-indanyl glycine, it should be possible to synthesize the required RRR DKP **13**. Reacting L-leucine **28** with benzaldehyde **7** and *t*-butylisonitrile **9** (Scheme 7) gave the SR dipeptide ester **29** as the major product (yield 87%). Hydrolysis of the ester **29** with lithium hydroxide in aqueous methanol gave a 55% yield of the acid **30**. Reaction of **30** via its mixed anhydride with *R-Boc*-indanyl glycine **10** gave **31**, which on cyclization gave the required RRR isomer **13** in a 47% yield, identical to that prepared via Scheme 1.

Conclusion

In summary, we have developed a short and novel stereospecific route to chirally pure RRR 13, SSS 24, and RSS 22 DKP's (the SRR could also be made by this route). We have established that the new chiral center produced in the major product from the 4-component-5-centered Ugi is opposite to that of the leucine component and that *R*-leucine gives RS 15 and *S*-leucine gives SR 29. This new chiral center then induces the stereochemistry of the leucine in the mixed anhydride reaction.

Experimental Procedures

(2*R*)-*N*-(*t*-Butyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-isobutyl-2,5-dioxopiperazin-1-yl]-2-phenylethanamide (13). To a solution of *R*-leucine methyl ester hydrochloride **6** (600 mg) in methanol (8 mL) was added triethylamine (0.46 mL) and benzaldehyde **7** (0.22 mL). The mixture was stirred for 2.5 h before (2*R*)-[(*t*-butoxycarbonyl)amino](2,3-dihydro-1*H*-inden-2-yl)ethanoic acid **10** (962 mg) and *t*-butylisonitrile **9** (0.56 mL) were sequentially added. After being stirred for 18 h, the solvent was removed in vacuo, and the residue was dissolved in dichloromethane (4 mL) and trifluoroacetic acid (10 mL) and stirred for 3 h at ambient temperature. After this time, the solvent was removed in vacuo. The residue was treated with triethylamine in dioxane (2% solution, 20 mL) and was left to stir overnight. After this time, the dioxane was removed in vacuo, and the residue was dissolved in dichloromethane. The solution was washed with 0.1 M hydrochloric acid solution, and the organic phase was separated using a hydrophobic frit and evaporated in vacuo. This crude material was purified by flash chromatography eluting with ethyl acetate/cyclohexane (50−100% ethyl acetate) to give the less polar diastereomer, (2*R*)-*N*-(*t*-butyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-isobutyl-2,5-dioxopiperazin-1-yl]-2-phenylethanamide (**13**) as a white solid (120 mg, 19%). ^{1}H NMR ($CDCl_3$) 7.45−7.38 (m, 5H), 7.24−7.13 (m, 4H), 6.72 (d, 1H, J = 4 Hz), 5.67 (s, 1H), 5.21 (s, 1H), 4.01−3.94 (m, 2H), 3.15 (m, 1H), 3.07 (m, 2H), 2.89 (m, 1H), 2.77 (m, 1H), 1.78 (m, 1H), 1.68 (m, 1H), 1.34 (m, 1H), 1.32 (s, 9H), 0.80 (d, 3H, J = 6.5 Hz), 0.71 (d, 3H, J = 6.5 Hz); ^{13}C NMR (CDCl3) 21.4, 23.9, 25.2, 28.9, 36.6, 37.2, 44.8, 46.3, 52.1, 59.4, 60.4, 65.8, 124.7, 125.0, 126.9, 127.1, 129.5, 129.6, 134.6, 141.6, 142.9, 167.1, 168.0, 169.3; LCMS *m/z* 476 (MH^+) (t_r 3.54 min); HRMS calcd for $C_{29}H_{37}N_3O_3$ (MH^+) 476.2913, found 476.2906; HPLC >99.5% (t_r = 14.3 min); circular dichroism λ_{max} 204.4, dE 21.49, λ_{max} 228.0, dE −15.60.

This also gave the more polar diastereomer, (2*S*)-*N*-(*t*-butyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-isobutyl-2,5-dioxopiperazin-1-yl]-2-phenylethanamide (**12**) as a white solid (622 mg, 40%) ^{1}H NMR ($CDCl_3$) 7.45−7.38 (m, 5H), 7.24−7.14 (m, 4H), 6.67 (d, 1H, J = 4 Hz), 5.80 (s, 1H), 5.42 (s, 1H), 3.98, (dd, 1H, J = 4,9.5 Hz), 3.75 (m, 1H), 3.19−3.02 (m, 3H), 2.96 (m, 1H), 2.77 (m, 1H), 1.91−1.65 (m, 3H), 1.36 (s, 9H), 0.80 (d, 3H, J = 6.5 Hz), 0.64 (d, 3H, J = 6.5 Hz);^{13}C NMR ($CDCl_3$) 21.3, 24.1, 25.2, 28.9, 36.7, 37.1, 44.2, 46.4, 52.0, 58.2, 60.3, 66.3, 124.7, 125.0, 126.9, 127.1, 129.5, 129.6, 130.2, 133.8, 141.6, 142.9, 167.3, 168.3, 169.3; LCMS *m/z* 476 (MH^+) (t_r 3.59 min); HRMS calcd for $C_{29}H_{37}N_3O_3$ (MH^+) 476.2913, found 476.2915; HPLC >99.5% (t_r =14.5 min)

Methyl (2*R*)-2-{[2-(*t*-Butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoate (15).

A stirred suspension of *R*-leucine **14** (1.31 g) in methanol (10 mL) under a nitrogen atmosphere was cooled to −30 °C.

To this was added a solution of benzaldehyde **7** (1.06 g) in methanol (0.5 mL) and *t*-butylisocyanide **9** (0.831 g). After 3 h at −30 °C, the reaction was allowed to warm to room temperature and was stirred for a further 72 h. The solvent was removed in vacuo, and the residue purified using a column (40 g, silica) eluting with cyclohexane/ethyl acetate (gradient from 8:1 to 1:1). The required fractions were combined and concentrated in vacuo to give methyl (2*R*)-2-{[2-(*t*-Butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoate (**15**) as a colorless oil (2.27, 68%). The ratio of isomers was 8:1, and the material was used without further purification.

Some of the mixture was purified to give the following isomers: major isomer ^{1}H NMR ($CDCl_3$) 7.37−7.27 (m, 5H), 6.51 (s, 1H), 4.11 (s, 1H), 3.71 (s, 3H), 3.11 (t, 1H, J = 7 Hz), 2.19 (broad signal, 1H), 1.70 (m, 1H), 1.48 (m, 2H), 1.31 (s, 9H), 0.87 (d, 3H, J = 6.5 Hz), 0.77 (d, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) 22.0, 22.8, 24.8, 28.6, 42.5, 50.9, 51.8, 57.5, 66.3, 127.8, 128.3, 128.9, 138.9, 170.8, 175.4; LCMS *m/z* 335 (MH^+) (t_r 3.38 min); HRMS calcd for $C_{19}H_{31}N_2O_3$ (MH^+) 335.233468, found 335.232462; chiral HPLC 99.5% (t_r 14.1 min).

Minor isomer ^{1}H NMR ($CDCl_3$) 7.38−7.27 (m, 6H), 3.96 (s, 1H), 3.72 (s, 3H), 3.32 (dd, 1H, J = 5, 8.5 Hz), 2.06 (broad signal, 1H), 1.87 (m, 1H), 1.50−1.42 (m, 2H), 1.39 (s, 9H), 0.98 (d, 3H, J = 6.5 Hz), 0.96 (d, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) 22.2, 23.2, 24.9, 28.7, 43.3, 50.7, 52.0, 59.0, 66.9, 127.2, 128.1, 128.8, 139.5, 170.9, 175.9; LCMS *m/z* 335 (MH^+) (t_r 3.42 min); HRMS calcd for $C_{19}H_{31}N_2O_3$ (MH^+) 335.233468, found 335.232511; chiral HPLC 100% (t_r 7.7 min)

Methyl (2*R*)-2-[[2-(*t*-Butylamino)-2-oxo-1-phenylethyl](formyl)amino]-4-methylpentanoate (16). To a solution of acetic anhydride (0.51 g) in dichloromethane (10 mL) was added formic acid (0.23 g) and pyridine (0.39 g), and this was stirred under nitrogen at ambient temperature for 1 h. To this was added a solution of methyl (2*R*)-2-{[2-(*t*-butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoate (**15**) (0.334 g) in dichloromethane (2 mL) and triethylamine (0.101 g), and the reaction mixture was left to stand under nitrogen at ambient temperature for 20 h. The reaction mixture was separated between dichloromethane and saturated sodium bicarbonate solution. The organic phase was washed with 2 N hydrochloric acid solution and brine. The organic phase was evaporated in vacuo to give methyl (2*R*)-2-[[2-(*t*-butylamino)-2-oxo-1-phenylethyl](formyl)amino]-4-methylpentanoate (**16**) as a white solid (0.274 g, 75%). ^{1}H NMR (d_6-DMSO) 8.24 (s, 1H), 7.99 (s, 1H), 7.49−7.27 (m, 6H), 5.24 (dd, 1H, J = 5.6,8.8 Hz), 3.70 (s, 3H), 1.83−1.46 (m, 3H), 1.31−1.20 (m, 9H), 0.90−0.75 (m, 6H); ^{13}C NMR (d_6-DMSO) 21.5, 22.9, 23.8, 28.1, 38.0, 50.7, 51.8, 52.1, 60.0, 127.8, 127.9, 128.7, 137.8, 163.9, 168.6, 171.7; LCMS *m/z* 363 (MH^+) (t_r 3.24 min); HRMS calcd for $C_{20}H_{30}N_2O_4$ (MH^+) 363.2284, found 363.2277; HPLC 93% (t_r = 13.0 min).

(2*R*)-2-{[2-(*t*-Butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoic Acid (17). Methyl (2*R*)-2-[[2-(*t*-butylamino)-2-oxo-1-phenylethyl]amino]-4-methylpentanoate (**15**) (0.59 g) was dissolved in methanol (15 mL), and to this was added lithium hydroxide (0.12 g) and water (2.5 mL). After 18 h, the methanol was removed in vacuo, and the residue was dissolved in water. The pH was adjusted to **7** using 2 N hydrochloric acid solution. This was applied to an OASIS cartridge (6 g) and eluted twice with water and twice with methanol. The methanol fractions were evaporated in vacuo to give (2*R*)-2-{[2-(*t*-butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoic acid (**17**) as a white solid (0.36 g, 64%).

Some of the mixture was purified to give the following isomers: major isomer ^{1}H NMR (d_6-DMSO) 7.63 (s, 1H), 7.37−7.21 (m, 5H), 4.12 (s, 1H), 2.80 (t, 1H, J = 7 Hz), 1.73 (m, 1H), 1.36 (m, 2H), 1.21 (s, 9H), 0.82 (d, 3H, J = 6.5 Hz), 0.70 (d, 3H, J = 6.5 Hz); ^{13}C NMR (d_6-DMSO) 21.9, 22.9, 24.3, 28.3, 42.2, 50.0, 57.6, 64.7, 127.0, 127.4, 127.9, 140.4, 171.0, 176.5; LCMS *m/z* 321 (MH^+) (t_r 2.46 min); chiral HPLC 100% (t_r 9.2 min); Anal. calcd. for $C_{18}H_{28}N_2O_3 \cdot 0.66\ H_2O$: C, 65.06; H, 8.89; N, 8.43, found: C, 65.16; H, 8.83; N, 8.36.

Minor isomer ^{1}H NMR (d_6-DMSO) 7.66 (s, 1H), 7.38−7.23 (m, 5H), 3.95 (s, 1H), 2.97 (t, 1H, J = 7 Hz), 1.84 (m, 1H), 1.39 (t, 2H, J = 7 Hz), 1.26 (s, 9H), 0.90 (d, 3H, J = 6.5 Hz), 0.89 (d, 3H, J = 6.5 Hz); ^{13}C NMR (d_6-DMSO) 22.1, 23.1, 24.4, 28.3, 42.5, 49.9, 58.9, 65.6, 127.3, 128.2, 140.2, 170.6, 176.2; LCMS *m/z* 321 (MH^+) (t_r 2.52 min); chiral HPLC 100% (t_r 7.1 min); Anal. calcd. for $C_{18}H_{28}N_2O_3 \cdot 0.33\ H_2O$: C, 66.24; H, 8.85; N, 8.58, found: C, 66.15; H, 8.62; N, 8.45.

(2*R*)-2-[[(1*S*)-2-(*t*-Butylamino)-2-oxo-1-phenylethyl](formyl)amino]-4-methylpentanoic Acid (19). To a solution of acetic anhydride (0.102 g) in dichloromethane (3 mL) was added formic acid (0.046 g) and pyridine (0.079 g), and the reaction mixture was stirred under nitrogen at ambient temperature for 20 min. To this was added (2*R*)-2-{[2-(*t*-butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoic acid (**17**) (0.10 g) and triethylamine (0.031 g). After 3 h, the reaction mixture was separated between dichloromethane and 1 N hydrochloric acid solution. The organic phase was dried using a hydrophobic frit and evaporated in vacuo to give (2*R*)-2-[[(1*S*)-2-(*t*-butylamino)-2-oxo-1-phenylethyl](formyl)amino]-4-methylpentanoic acid (**19**) as an off-white solid (0.108 g, 100%). ^{1}H NMR (d_6-DMS0) 13.10 (s, 1H), 8.29 (s, 1H), 7.44 (m, 1H), 7.38 (m, 1H), 7.32 (m, 1H), 5.34 (s, 1H), 5.15 (dd, 1H, J = 5,9 Hz), 1.70−1.51 (m, 3H), 1.31 (s, 9H), 0.87 (d, 3H, J = 6 Hz), 0.86 (d, 3H, J = 6 Hz); ^{13}C NMR 20.2, 21.6, 22.5, 26.8, 36.9, 49.3, 50.6, 58.7, 126.4, 126.5, 127.2, 136.7, 162.6, 167.6, 171.6; LCMS *m/z* 349 (MH^+) (t_r 3.13 min); HRMS calcd for $C_{19}H_{29}N_2O_4$ (MH^+) 349.2127, found 349.2127; HPLC 99% (t_r = 12.0 min).

Methyl (2*R*)-2-[[(1*S*)-2-(*t*-Butylamino)-2-oxo-1-phenylethyl](formyl)amino]-4-methylpentanoate (16). To a solution of (2*R*)-2-[[(1*S*)-2-(*t*-butylamino)-2-oxo-1-phenylethyl](formyl)amino]-4-methylpentanoic acid (**19**) (36 mg) in tetrahydrofuran (2 mL) was added a 2.0 M solution of trimethylsilyldiazomethane in hexanes (0.25 mL), and the reaction mixture was stirred under nitrogen at ambient temperature for 2 h. Methanol (2 mL) was added, and the reaction mixture was stirred for a further 2 h. The solvent was removed in vacuo, and the residue was purified by mass directed autoprep. This gave methyl (2*R*)-2-[[(1*S*)-2-(*t*-butylamino)-2-oxo-1-phenylethyl](formyl)amino]-4-methylpentanoate (**16**) as an off-white solid (30 mg, 80%).

(2*S*)-*N*-(*t*-Butyl)-2-[(3*R*,6*S*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-isobutyl-2,5-dioxopiperazin-1-yl]-2-phenylethanamide (22). To a solution of (2*R*)-[(*t*-butoxycarbonyl)amino](2,3-dihydro-1*H*-inden-2-yl)ethanoic acid (**10**) (58 mg) in dry tetrahydrofuran (3 mL) under a nitrogen atmosphere at −25 °C was added *N*-methylmorpholine (20 mg) and a solution of isopropylchloroformate in toluene (1.0 M, 0.2 mL). After 10 min, (2*R*)-2-{[2-(*t*-butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoic acid (**17**) (70 mg) was added, and the resultant mixture was stirred at room temperature for 3 h. The solvent was then removed in vacuo, and the residue was treated with 4 N hydrochloric acid in dioxane (8 mL). After 4 h, methanol (15 mL) was added to the reaction mixture, and this was left to stand for 18 h. The solvent was then removed in vacuo, and the residue was purified on an SPE cartridge (50 g, silica) eluting with cyclohexane/ethyl acetate (gradient from 4:1 to neat ethyl acetate), which furnished (2*S*)-*N*-(*t*-butyl)-2-[(3*R*,6*S*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-isobutyl-2,5-dioxopiperazin-1-yl]-2-phenylethanamide (**22**) as a white solid (54 mg, 57%) ^{1}H NMR ($CDCl_3$) d 7.42 (m, 5H), 7.20 (m, 2H), 7.17 (m, 2H), 5.64 (s, 1H), 5.59 (s, 1H), 5.48 (s, 1H), 4.23 (d, 1H, J = 4 Hz), 4.00 (m, 1H), 3.39 (m, 2H), 3.13 (m, 1H), 2.91−2.79 (m, 2H), 1.63−1.51 (m, 2H), 1.33 (s, 9H), 0.87 (m, 1H), 0.71 (d, 3H, J = 6 Hz), 0.58 (d, 3H, J = 6 Hz); ^{13}C NMR ($CDCl_3$) 20.9, 23.4, 24.2, 28.6, 34.1, 36.3, 38.6, 40.8, 51.8, 57.6, 58.7, 63.3, 124.5, 124.8, 126.7, 126.9, 129.17, 129.19, 129.9, 134.2, 141.4, 142.0, 167.5, 167.6, 169.5; LCMS *m/z* 476 (MH^+) (t_r 3.55 min); HRMS calcd for $C_{29}H_{37}N_3O_3$ (MH^+) 476.2913, found 476.2908; HPLC 96% (t_r = 14.4).

(2*S*)-*N*-(*t*-Butyl)-2-[(3*S*,6*S*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-isobutyl-2,5-dioxopiperazin-1-yl]-2-phenylethanamide (24). To a solution of (2*S*)-[(*t*-butoxycarbonyl)amino](2,3-dihydro-1*H*-inden-2-yl)ethanoic acid (**23**) (58 mg) in dry tetrahydrofuran (3 mL) under a nitrogen atmosphere at −25 °C was added *N*-methylmorpholine (20 mg) and a solution of

isopropylchloroformate in toluene (1.0 M, 0.2 mL). After 10 min, (2*R*)-2-{[2-(*t*-butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoic acid (**17**) (64 mg) was added, and the resultant mixture was stirred at room temperature for 3 h. The solvent was then removed in vacuo, and the residue was treated with 4 N hydrochloric acid in dioxane (8 mL). After 3 h, the solvent was removed in vacuo, and the residue was dissolved in dichloromethane (2 mL). To this was added diisopropylethylamine (78 mg) and WSCDI (58 mg), and the reaction mixture was left to stand for 18 h. The solvent was then removed in vacuo, and the residue was purified on an SPE cartridge (50 g, silica) eluting with cyclohexane/ethyl acetate (gradient from 4:1 to neat ethyl acetate), which furnished (2*S*)-*N*-(*t*-butyl)-2-[(3*S*,6*S*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-isobutyl-2,5-dioxo piperazin-1-yl]-2-phenylethanamide (**24**) as a white solid (34 mg, 36%). ^{1}H NMR ($CDCl_3$) 7.47−7.37 (m, 6H), 7.24−7.13 (m, 4H), 5.78 (s, 1H), 5.23 (s, 1H), 4.01−3.94 (m, 2H), 3.17−2.98 (m, 3H), 2.94−2.77 (m, 2H), 1.78 (m, 1H), 1.67 (m, 1H), 1.38−1.29 (m, 10H), 0.81 (d, 3H, J = 6.5 Hz), 0.72 (d, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) 21.4, 23.9, 25.2, 28.9, 36.6, 37.2, 44.8, 46.3, 52.1, 59.4, 60.4, 65.8, 124.7, 125.0, 126.9, 127.1, 129.5, 129.6, 134.6, 141.6, 142.9, 167.1, 168.0, 169.3; LCMS *m/z* 476 (MH^+) (t_r 3.38 min); HRMS calcd for $C_{29}H_{37}N_3O_3$ (MH^+) 476.2913, found 476.2903; HPLC >99.5% (t_r =14.3 min); circular dichroism λ_{max} 204.4, dE −21.61, λ_{max} 228.0, dE 15.30.

To a solution of (2*R*)-[(*t*-butoxycarbonyl)amino](2,3-dihydro-1*H*-inden-2-yl)ethanoic acid (**10**) (58 mg) in dry tetrahydrofuran (3 mL) under a nitrogen atmosphere at −25 °C was added *N*-methylmorpholine (20 mg) and a solution of isopropylchloroformate in toluene (1.0 M, 0.2 mL). After 10 min, (2*R*)-2-{-[2-(*t*-butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoic acid (**17**) (64 mg) was added, and the resultant mixture was stirred at between −20 and −15 °C for 2 h. The solvent was then removed in vacuo, and the residue was treated with 4 N hydrochloric acid in dioxane (8 mL). After 3 h, the solvent was removed in vacuo, and the residue was dissolved in dichloromethane (2 mL). To this was added diisopropylethylamine (78 mg) and WSCDI (58 mg), and the reaction mixture was left to stand for 18 h. The solvent was then removed in vacuo, and the residue was purified on an SPE cartridge (50 g, silica) eluting with cyclohexane/ethyl acetate (gradient from 4:1 to neat ethyl acetate), which furnished (2*S*)-*N*-(*t*-butyl)-2-[(3*R*,6*S*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-isobutyl-2,5-dioxopiperazin-1-yl]-2-phenylethanamide (**22**) as a white solid (26 mg, 27%) and (2*S*)-*N*-(*t*-butyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-isobutyl-2,5-dioxopiperazin-1-yl]-2-phenylethanamide (**12**) as a white solid (14 mg, 15%).

Methyl (2*S*)-2-{[2-(*t*-Butylamino)-2-oxo-1-phenylethyl]-amino}-4-methylpentanoate (29). A stirred suspension of *S*-leucine (**28**) (2.62 g) in methanol (200 mL) under a nitrogen atmosphere was cooled to −30 °C. To this was added a solution of benzaldehyde (**7**) (2.12 g) in methanol (10 mL) and a solution of *t*-butylisocyanide (**9**) (1.66 g) in methanol (10 mL). After 4 h at −30 °C, the reaction was allowed to warm to room temperature and was stirred for a further 72 h. The solvent was removed in vacuo, the residue was purified using a column (40 g, silica) eluting with cyclohexane/ethyl acetate (gradient from 8:1 to 1:1). The required fractions were combined and concentrated in vacuo to give methyl (2*S*)-2-{[2-(*t*-butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoate (**29**) as a colorless oil (5.82 g, 87%). The ratio of isomers was 9:1, and material used without further purification.

Some of the mixture was purified to give the following isomers: major isomer ^{1}H NMR ($CDCl_3$) 7.37−7.27 (m, 5H), 6.52 (s, 1H), 4.12 (s, 1H), 3.71 (s, 3H), 3.12 (t, 1H, J = 7 Hz), 1.70 (m, 1H), 1.48 (m, 2H), 1.31 (s, 9H), 0.87 (d, 3H, J = 6.5 Hz), 0.77 (d, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) 22.0, 22.8, 24.8, 28.6, 42.5, 50.1, 51.8, 57.5, 66.3, 127.8, 128.3, 128.9, 138.8, 170.8, 175.4; LCMS *m/z* 335 (MH^+) (t_r 3.40 min); HRMS calcd for $C_{19}H_{31}N_2O_3$ (MH^+) 335.233468, found 335.232467; chiral HPLC 99.5% (t_r 7.0 min).

Minor isomer ^{1}H NMR ($CDCl_3$) 7.38−7.27 (m, 6H), 3.96 (s, 1H), 3.72 (s, 3H), 3.32 (dd, 1H, J = 5,8.5 Hz), 2.06 (broad signal, 1H), 1.87 (m, 1H), 1.50−1.42 (m, 2H), 1.39 (s, 9H), 0.98 (d, 3H, J = 6.5 Hz), 0.96 (d, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) 22.2, 23.2, 24.9, 28.7, 43.3, 50.7, 52.0, 59.0, 66.9, 127.2, 128.1, 128.8, 139.5, 170.9, 175.9; LCMS *m/z* 335 (MH^+) (t_r 3.46 min); HRMS calcd for $C_{19}H_{31}N_2O_3$ (MH^+) 335.233468, found 335.234355; chiral HPLC 100% (t_r 13.7 min).

(2*S*)-2-{[2-(*t*-Butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoic acid (30). Methyl (2*S*)-2-[[2-(*t*-butylamino)-2-oxo-1-phenylethyl]amino]-4-methylpentanoate (**29**) (1.32 g) was dissolved in methanol (15 mL), and to this was added lithium hydroxide (0.29 g) and water (10 mL). After 4 h, the methanol was removed in vacuo, and the residue was dissolved in water. The pH was adjusted to 7 using a 2 N hydrochloric acid solution. This was applied to an OASIS cartridge (6 g) and eluted twice with water and twice with methanol. The methanol fractions were evaporated in vacuo to give (2*S*)-2-{[2-(*t*-butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoic acid (**30**) as a white solid (0.36 g, 55%).

Some of the mixture was purified to give the following isomers: major isomer ^{1}H NMR (d_6-DMSO) 7.65 (s, 1H), 7.37−7.20 (m, 5H), 4.11 (s, 1H), 2.79 (dd, 1H, J = 6,7 Hz), 1.74 (m, 1H), 1.36 (m, 2H), 1.21 (s, 9H), 0.82 (d, 3H, J = 6.5 Hz), 0.70 (d, 3H, J = 6.5 Hz); ^{13}C NMR (d_6-DMSO) 22.0, 23.0, 24.3, 28.4, 42.3, 50.0, 57.8, 64.8, 127.1, 127.5, 128.0, 140.6, 171.5, 176.5; LCMS *m/z* 321 (MH^+) (t_r 2.46 min); chiral HPLC 100% (t_r 7.8 min); Anal. calcd. for $C_{18}H_{28}N_2O_3 \cdot 0.5\ H_2O$: C, 65.63; H, 8.87; N, 8.50, found: C, 65.58; H, 8.56; N, 8.40.

Minor isomer ^{1}H NMR (d_6-DMSO) 7.66 (s, 1H), 7.38−7.23 (m, 5H), 3.96 (s, 1H), 2.98 (t, 1H, J = 7 Hz), 1.84 (m, 1H), 1.39 (t, 2H, J = 7 Hz), 1.26 (s, 9H), 0.90 (d, 3H, J = 6.5 Hz), 0.89 (d, 3H, J = 6.5 Hz); ^{13}C NMR (d_6-DMSO) 22.1, 23.1, 24.4, 28.3, 42.5, 49.9, 58.8, 65.5, 127.3, 127.4, 128.2, 140.1, 170.6, 176.1; LCMS *m/z* 321 (MH^+) (t_r 2.52 min); chiral HPLC 100% (t_r 5.5 min); Anal. calcd. for $C_{18}H_{28}N_2O_3 \cdot 0.33\ H_2O$: C, 66.24; H, 8.85; N, 8.58, found: C, 66.36; H, 8.66; N, 8.48.

(2*R*)-*N*-(*t*-Butyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-isobutyl-2,5-dioxopiperazin-1-yl]-2-phenylethanamide (13). To a solution of (2*R*)-[(*t*-butoxycarbonyl)amino](2,3-dihydro-1*H*-inden-2-yl)ethanoic acid (**10**) (58 mg) in dry tetrahydrofuran (3 mL) under a nitrogen atmosphere at −25 °C was added *N*-methylmorpholine (20 mg) and a solution of isopropylchloroformate in toluene (1.0 M, 0.2 mL). After 10 min, (2*S*)-2-{[2-(*t*-butylamino)-2-oxo-1-phenylethyl]amino}-4-methylpentanoic acid (**30**) (70 mg) was added, and the resultant mixture was stirred at room temperature for 3 h. The solvent was then removed in vacuo, and the residue was treated with 4 N hydrochloric acid in dioxane (8 mL). After 4 h, methanol (15 mL) was added to the reaction mixture, and this was left to stand for 18 h. The solvent was then removed in vacuo, and the residue was purified on an SPE cartridge (50 g, silica) eluting with cyclohexane/ethyl acetate (gradient from 4:1 to neat ethyl acetate), which furnished (2*R*)-*N*-(*t*-butyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-isobutyl-2,5-dioxopiperazin-1-yl]-2-phenylethanamide (**13**) as a white solid (45 mg, 47%) ^{1}H NMR ($CDCl_3$) 7.45−7.38 (m, 5H), 7.24−7.14 (m, 4H), 6.38 (m, 1H), 5.64 (s, 1H), 5.19 (s, 1H), 3.97 (m, 2H), 3.20−3.01 (m, 3H), 2.91 (m, 1H), 2.77 (m, 1H), 1.91−1.65 (m, 2H), 1.32 (m, 10H), 0.79 (d, 3H, J = 6.5 Hz), 0.71 (d, 3H, J = 6.5 Hz); LCMS *m/z* 476 (MH^+) (t_r 3.59 min).

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Supporting Information Available: ^{1}H NMR for isolated compounds. Circular dichroism spectra for **13** and **24**. This material is available free of charge via the Internet at http://pubs.acs.org.

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