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Synthesis and Cytotoxicity of the Proposed Structure of Piperazirum, Its Stereoisomers and Analogues

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Piperazirum, a new biologically active alkaloid isolated from *Arum palaestinum* Bioss, is described, starting from commercially available α-amino acids. Two synthetic strategies were developed for the synthesis of the enantiomeric pair of compounds corresponding to the proposed structure of piperazirum. The first strategy was used in the synthesis of stereoisomers, and the second one was used in generating the analogues. The compounds synthesized were screened against different cell lines to evaluate their cytotoxicity profiles.

Introduction

The piperazine and piperazinone motifs are considered to be privileged structures in drug discovery.[1] Such motifs are found in the structures of several natural products that show a wide range of biological activities, such as pseudotheonamides A_1, A_2, and B_3,[2] dragmacidin[3] and dragmacidins A–C,[4] dihydrohamacanthin A,[5] ecteinascidin 743,[6] TAN1251A,[7] agelastatin A,[8] marcfortine B,[9] phakellins,[10] and guadinomine C_2.[11] This ring is also present in compounds with antifungal,[12] antidepressant,[13] antimigraine,[14] antithrombotic,[15] antihistaminic,[16] antiaggregating,[17] or nootropic[18] activities. In 2007, piperazirum, a new biologically active alkaloid, was isolated from the *n*-butanol-soluble fraction of the leaf extract o *Arum palaestinum* Bioss.[19] It was found to show significant inhibition of all the cultured human cell lines examined using the SRB (sulforhodamine B) method.[20] These cell lines included A549 (non-small cell lung, $ED_{50} = 4.26 \pm 0.2$ μM), SK-OV-3 (ovary, $ED_{50} = 1.38 \pm 0.1$ μM), SK-MEL-2 (melanoma, $ED_{50} = 0.51 \pm 0.1$ μM), and HCT-15 (colon, $ED_{50} = 2.47 \pm 0.3$ μM). The structure and relative stereochemistry of piperazirum were deduced on the basis of 1D and 2D NMR spectroscopic data. To the best of our knowledge, only two reports of the synthesis of this compound have been published.[21] The first one describes a synthetic route to 2-oxopiperazine derivatives targeting the natural product piperazirum,[21a] and the second one, which appeared very recently, describes the synthesis of the reported structure of piperazirum.[21b] However, the correct structure and the absolute configuration of this compound are not yet known.

The unknown absolute stereochemistry, the highly substituted chiral 2-oxopiperazine structure, and the promising cytotoxicity of piperazirum attracted us to develop a strategy for its synthesis. We aimed to develop a strategy for the synthesis of this molecule that would not only provide enough material for further biological evaluation of piperazirum, but that would also enable access to stereoisomers and analogues that could be used to study a structure–activity relationship (SAR) and so find more potent molecules. In this paper, we describe the synthesis of the proposed structure of piperazirum, and also its stereoisomers and analogues. We also give the results of testing their in vitro antiproliferative activity against seven different cell lines, including A549 (human lung adenocarcinoma epithelial cell line), SK-OV-3 (ovarian carcinoma cell line), DU-145 (human prostate cancer cell line), MDA-MB-231 and MCF-7 (human breast cancer cell lines), HEK-293 (human embryonic kidney cells), and NIH/3T3 (mouse embryo fibroblast cell line).

Results and Discussion

Our retrosynthetic analysis of **1** is shown in Figure 1. To get the *syn* stereochemistry at C-3, C-5, and C-6, we chose acyclic amide **2**, in which the amine, upon deprotection of the Boc (*tert*-butoxycarbonyl) group, could displace the leaving group (X) in an S_N2 fashion to give the target molecule under basic conditions. Acyclic compound **2** could

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easily be derived from the coupling of 1,2-diamine derivative **3** and α-hydroxy acid **4**. These two fragments could be made from the same starting material, L-leucine.

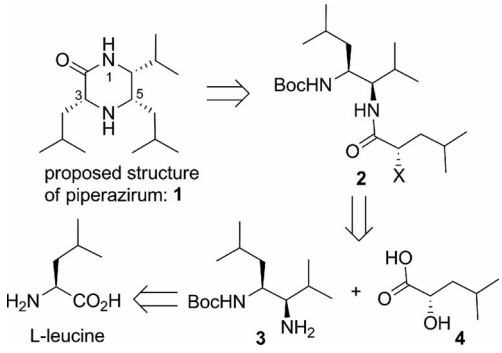
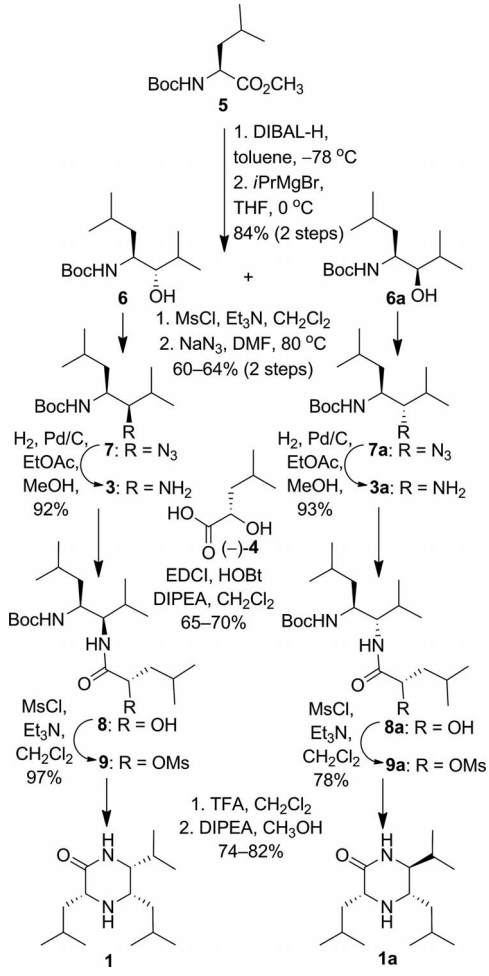


Figure 1. Retrosynthetic analysis of the proposed structure of piperazirum (**1**).

The synthesis began with a reduction of **5**[22] using DIBAL-H (diisobutylaluminium hydride), to convert the ester into an aldehyde (Scheme 1). The aldehyde was treated with isopropylmagnesium bromide to give a separable mixture of diastereomers **6** and **6a** (3.5:1 ratio) in 85% combined yield. Mesylation of **6**, the major isomer, with MsCl and Et_3N in CH_2Cl_2 followed by displacement of the mesyl group with sodium azide gave the corresponding amino azide (i.e., **7**) in 83% yield. Hydrogenation of **7** under balloon pressure in the presence of Pd/C in a mixture of EtOAc/MeOH (2:1) gave amine **3** in quantitative yield. Primary amine **3** was coupled to α-hydroxy acid (–)-**4**, which had been derived from L-leucine by the diazotization method,[23] in the presence of EDCI [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide]/HOBt to give amide **8**. The free hydroxy group in **8** was mesylated to give **9**. Finally, removal of the Boc protecting group with trifluoroacetic acid (TFA), followed by heating at reflux in the presence of DIPEA (diisopropylethylamine) gave the desired compound (i.e., **1**). We could not compare the NMR spectroscopic data of synthetic **1** with the data reported for the natural product due to the insolubility of the synthetic sample in D_2O, the solvent for which the NMR spectroscopic data of the natural product was reported.[24]

With this solubility discrepancy in mind, we speculated that the substituents at C-3, C-5, and C-6 on the piperazine ring may not have the *syn* relationship proposed by the isolation group.[19] We thought that stereoisomers in which the substituents had an *anti* relationship may differ in their solubilities, and so the synthesis of these compounds may help to find the absolute configuration of piperazirum.

Compound **6a** was mesylated, and subsequent displacement of the mesyl group with sodium azide gave the corresponding amino azide (i.e., **7a**; Scheme 1). Azide **7a** was treated with hydrogen at atmospheric pressure in the presence of Pd/C in a mixture of EtOAc/MeOH (2:1) to give **3a**. Under standard peptide-coupling conditions, **3a** was coupled with acid (–)-**4** to give **8a**. The hydroxy group in **8a** was mesylated with MsCl/Et_3N, and subsequent removal of the Boc protecting group followed by heating at reflux in

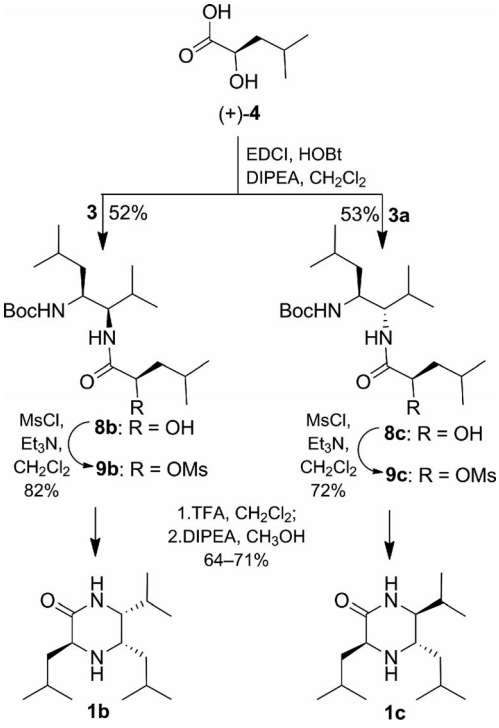


Scheme 1. Synthesis of **1** and **1a**.

the presence of DIPEA in MeOH resulted the formation of the expected cyclic product (i.e., **1a**).

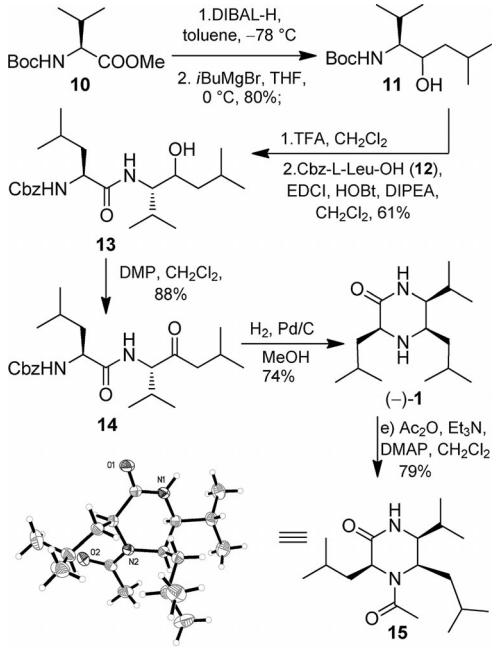
Other stereoisomers were planned from coupling of **3** and **3a**, independently, with α-hydroxy acid (+)-**4**,[25] derived from D-leucine, to give **8b** and **8c**, respectively (Scheme 2). Treatment of **8b** and **8c** with mesyl chloride under basic conditions gave **9b** and **9c**, respectively. Upon Boc group deprotection and subsequent heating at reflux in MeOH in the presence of DIPEA, **9b** and **9c** provided **1b** and **1c**, respectively. To our surprise, none of these isomers (**1a**–**1c**) was soluble in D_2O, so we could not compare the spectroscopic data with that of the natural product, which hindered the confirmation of the structure as well as finding the absolute configuration of the natural product.[24b]

As piperazirum was reported to have significant cytotoxicity against cancer cell lines,[19] we expected that the synthesis of analogues of **1** could result in our finding potent molecules based on a structure–activity relationship. Working towards this end, we envisioned another strategy for the synthesis of the enantiomer of **1** (*ent*-**1**), in order to access analogues of **1** or its enantiomer by a shorter and more efficient route. This synthesis started with the reduction of **10**[26] using DIBAL-H, to convert the methyl ester into the aldehyde. This aldehyde was then treated with isobutylmag-



Scheme 2. Synthesis of **1b** and **1c**.

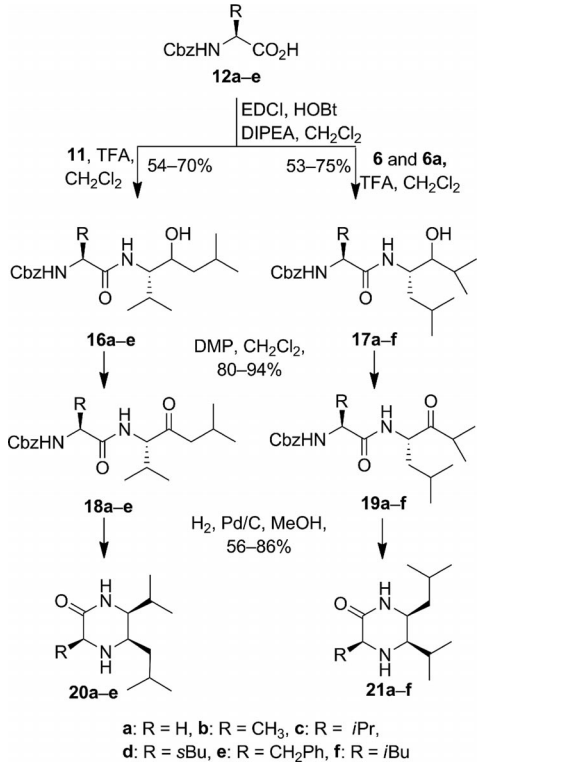
nesium bromide to give **11** (*dr* 7:1, based on ^{1}H NMR spectroscopy; Scheme 3). Deprotection of the Boc protecting group provided the free amine, which was coupled with **12** (Cbz-L-Leu-OH; Cbz = benzyloxycarbonyl)[27] in the presence of EDCI/HOBt to give dipeptide **13**. Oxidation of the secondary hydroxy group into a ketone using Dess–Martin periodinane (DMP) produced **14**. Finally, **14** was treated



Scheme 3. Synthesis of (–)-**1** and **15**; DMAP = 4-(dimethylamino)-pyridine.

with hydrogen at atmospheric pressure in the presence of Pd/C in MeOH. The Cbz group was removed, and the imine formed intramolecularly was saturated to give the anticipated *ent*-**1** in excellent yield and with excellent diastereoselectivity. The spectroscopic data of **1** and *ent*-**1** are identical in all respects.[21b] The specific rotations of **1** and *ent*-**1** have the same magnitude but opposite signs, which indicates, as expected, that the two compounds have an enantiomeric relationship. The *syn* configuration of **1** and *ent*-**1** was further confirmed by the X-ray analysis of **15**,[28] derived from (–)-**1**, which made it clear that **1** or *ent*-**1** is the proposed structure of piperazirum. Comparison of the optical rotation of all of the synthesized stereoisomers with the natural product showed that only **1** {$[\alpha]_D^{26}$ = +52.5 (*c* = 0.33, MeOH)} had an optical rotation that was very close to the one reported for natural product {$[\alpha]_D$ = +0.0 (*c* = 0.024, MeOH)},[19] which indicates that **1** may be the correct structure for piperazirum. However, comparing the NMR spectroscopic data was difficult because of the uncertainty over the solvent (D_2O or any other) used to record the NMR spectra of the natural product.[24b]

After the successful completion of the short and efficient synthetic route for rapid access to *ent*-**1**, we focussed our attention on the synthesis of some higher analogues using the same strategy. Accordingly, the free amine derived from **11** was coupled with **12a**–**12e** [Cbz-L-CH(R)-OH, a: R = H; b: R = CH_3;[29] c: R = *i*Pr; d: R = *sec*-Bu;[30] e: R = CH_2Ph] to give **16a**–**16e**. Similarly, the free amine derived from **6** and **6a** was coupled with **12a**–**12e** and **12** [Cbz-L-CH(R)-OH, R = *i*Bu] to give **17a**–**17e** and **17f**, respectively (Scheme 4). DMP oxidation of **16a**–**16e** and **17a**–**17f** gave



Scheme 4. Synthesis of **20a**–**20e** and **21a**–**21f**.

Table 1. In-vitro anticancer activity (IC_{50} [μM]) of the proposed structure of piperazirum (+)-**1**, its enantiomer (–)-**1**, its diastereomers **1a**–**1c**, and its analogues (**20a**–**20e**, **21a**–**21f**).

	A549	SK-OV-3	DU-145	MDA-MB-231	MCF-7	HEK-293	NIH/3T3
(+)-**1**	25 ± 0.37	19 ± 0.37	45 ± 0.24	50 ± 0.23	40 ± 0.22	59 ± 0.20	61 ± 0.01
(–)-**1**	21 ± 0.15	17 ± 0.92	36 ± 0.21	40 ± 0.27	26 ± 0.22	53 ± 0.26	63 ± 0.16
1a	77 ± 0.39	43 ± 0.91	81 ± 0.43	78 ± 0.19	86 ± 0.13	95 ± 0.10	89 ± 0.16
1b	62 ± 0.55	54 ± 0.76	73 ± 0.17	77 ± 0.07	69 ± 0.31	69 ± 0.46	78 ± 0.66
1c	66 ± 0.2	42 ± 0.19	92 ± 0.10	87 ± 0.19	90 ± 0.12	98 ± 0.17	89 ± 0.16
20a	37 ± 0.86	18 ± 0.51	61 ± 0.20	63 ± 0.24	67 ± 0.24	63 ± 0.22	61 ± 0.21
20b	16 ± 0.46	9.3 ± 0.39	22 ± 0.26	19 ± 0.24	28 ± 0.54	48 ± 0.14	52 ± 0.12
20c	71 ± 0.77	28 ± 0.62	80 ± 0.16	82 ± 0.16	89 ± 0.12	87 ± 0.19	83 ± 0.29
20d	51 ± 0.7	59 ± 0.47	77 ± 0.32	67 ± 0.49	89 ± 0.42	89 ± 0.02	81 ± 0.12
20e	7 ±0.33	4.4 ± 0.13	10.5 ± 0.2	19.2 ± 0.1	14 ± 0.3	34 ± 0.23	37 ± 0.28
21a	86 ± 0.78	57 ± 0.69	93 ± 0.29	91 ± 0.45	96 ± 0.21	92 ± 0.76	88 ± 0.36
21b	70 ± 0.97	46 ± 0.58	89 ± 0.12	90 ± 0.52	99 ± 0.32	87 ± 0.37	83 ± 0.34
21c	44 ± 0.98	52 ± 0.57	72 ± 0.35	71 ± 0.25	78 ± 0.45	76 ± 0.40	71 ± 0.20
21d	82 ± 0.52	90 ± 0.58	81 ± 0.59	89 ± 0.54	95 ± 0.50	95 ± 0.50	81 ± 0.59
21e	32 ± 0.69	17 ± 0.86	51 ± 0.63	52 ± 0.63	55 ± 0.65	59 ± 0.05	52 ± 0.15
21f	43 ± 0.61	41 ± 0.41	61 ± 0.61	69 ± 0.57	62 ± 0.87	60 ± 0.17	65 ± 0.11

the corresponding ketones (i.e., **18a**–**18e** and **19a**–**19f**, respectively). Removal of the Cbz protecting group from **18a**–**18e** and **19a**–**19f** and saturation of the subsequently formed imine under hydrogenation conditions gave substituted chiral 2-oxopiperazines **20a**–**20e** and **21a**–**21f**, respectively.

The proposed structure of piperazirum (+)-**1**/(–)-**1**, its diastereoisomers **1a**–**1c**, and its analogues **20a**–**20e**, and **21a**–**21f** were tested for their antiproliferative activity, and the results are summarized in Table 1.

Biological Studies: Anti-Proliferative Activity

The proposed structure of piperazirum (+)-**1**/(–)-**1**, its diastereoisomers **1a**–**1c**, and its analogues **20a**–**20e**, and **21a**–**21f** were evaluated for in vitro anticancer activity using an MTT assay across different concentrations (2.5, 5, 10, 20, 50, and 100 μM) in triplicate. The growth-inhibitory effects were studied in five human cancer cell lines, i.e., A549, SK-OV-3, DU-145, MDA-MB-231, and MCF-7, and two non-cancer cell lines, i.e., HEK-293 and NIH/3T3. Compound **1** has IC_{50} values of 25, 19, 45, 50, 40, 59 and 61 μM against A549, SK-OV-3, DU-145, MDA-MB-231, MCF-7, HEK-293, and NIH/3T3, respectively (Table 1). Compound (–)-**1** also showed similar in vitro cytotoxic activity (Table 1) against all the cell lines tested, and this enantiomer showed the highest activity against SK-OV-3 cells, with an IC_{50} value of 17 μM. It is interesting to note that derivatives **20a**–**20e** significantly inhibited the growth of ovarian cancer cell line (SK-OV-3) with IC_{50} values of 18 μM (**20a**), 9 μM (**20b**), 28 μM (**20c**), 59 μM (**20d**), and 4.4 μM (**20e**). Compound **20e** showed significant cytotoxicity against all the cancer cell lines tested, with IC_{50} values of 7, 4.4, 10.5, 19.2, and 14 μM against A549, SK-OV-3, DU-145, MDA-MB-231, and MCF-7, respectively. Moreover, compound **20e** showed selective cytotoxicity towards cancer cells. Similarly, of analogues **21a**–**21f**, **21e** (17 μM) has a better activity profile specifically against SK-OV-3 cells than **21a** (57 μM), **21b** (46 μM), **21c** (52 μM), **21d** (90 μM), and **21f** (41 μM). None of the stereoisomers **1a**–**1c** showed significant cytotoxicity against any of the cancer cell lines, except for **1a** (43 μM) and **1c** (42 μM), which have some antiproliferative activity specifically against the SK-OV-3 cancer cell line.

Conclusions

In conclusion the enantiomeric pair corresponding to the proposed structure of piperazirum was synthesized using two different synthetic strategies. Stereoisomers and analogues were synthesized following these strategies, and all the compounds were evaluated for in vitro anticancer activity against different cancer cell lines. X-ray analysis of **15** supports that **1** or *ent*-**1** is the proposed structure of piperazirum. The enantiomer of the proposed structure of piperazirum and its analogues showed significant cytotoxicity against ovarian cancer cell lines. Interestingly, **20e** showed selective anticancer activity against all the cancer cell lines tested.

Experimental Section

General Procedures: Anhydrous solvents were dried and distilled by standard methods before use. Commercially sourced reagents were used without further purification unless otherwise specified. All reactions were performed under an atmosphere of nitrogen or argon in oven-dried glassware with magnetic stirring. Column chromatography was carried out using silica gel (60–120, 100–200, or 230–400 mesh) and the column was eluted with ethyl acetate/petroleum ether or with ethyl acetate. Analytical thin layer chromatography (TLC) was performed on precoated silica gel-60 F254 (0.5 mm) glass plates. Visualization of the spots on TLC plates was achieved either using UV light or by staining the plates with ninhydrin/methanol, methanolic anisaldehyde/sulfuric acid/acetic acid, or methanol/phosphomolybdic acid/sulfuric acid solutions followed by charring on a hot plate. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ with 500, 400, and 300 MHz spectrometers at ambient temperature. Chemical shifts are reported on the δ scale, and were calibrated using internal $CHCl_3$ (δ = 7.26 ppm) or tetramethylsilane (δ = 0.0 ppm) for ^1H NMR spectra, and $CHCl_3$ (δ = 77.0 ppm) for

^{13}C NMR spectra. ^{1}H NMR spectroscopic data is recorded as follows: chemical shift {multiplicity, coupling constant(s) *J* [Hz], relative integral} where multiplicity is defined as: s = singlet; d = doublet; t = triplet; q = quartet; dd = doublet of doublets; dt = doublet of triplets; m = multiplet; br. s = broad singlet; br. d = broad doublet. FTIR spectra were recorded with a Bruker (Alpha) spectrometer. Optical rotation values were measured with a Horiba high-sensitivity polarimeter using a 2 mL cell with a 10 mm path length. Mass spectra were recorded with a Micromass VG-7070H mass spectrometer using the ESI technique and are reported in mass units (*m*/*z*). High-resolution mass spectra (HRMS, ESI) were obtained using either a TOF or a double-focussing spectrometer.

Methyl (*S*)-2-(*tert*-Butoxycarbonylamino)-4-methylpentanoate (5):[22] Thionyl chloride (5.56 mL, 76.231 mmol) was added dropwise to a solution of L-leucine (10 g, 76.231 mmol) in dry MeOH (100 mL) at 0 °C under a nitrogen atmosphere. The resulting mixture was stirred at room temp. for 2 h, and then it was heated at reflux for 8 h. Then the reaction mixture was allowed to cool to room temp., and the solvent was evaporated under reduced pressure to give the methyl ester as a solid.

The methyl ester was dissolved in dry CH_2Cl_2 (110 mL) under N_2 at 0 °C, and Et_3N (21.23 mL, 152.462 mmol) was added, followed by Boc anhydride (19.24 mL, 83.854 mmol). The reaction mixture was stirred at room temp. overnight. Water was added to the reaction mixture, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography to give Boc-L-Leu-OMe (**5**) (18.5 g, 97%) as a colourless oil. R_f = 0.5 (15% EtOAc/hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.85 (br. d, *J* = 8.3 Hz, 1 H), 4.25 (m, 1 H), 3.70 (s, 3 H), 1.68 (m, 1 H), 1.60–1.46 (m, 2 H), 1.41 (s, 9 H), 0.99–0.88 (m, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.9, 155.3, 79.7, 52.1, 51.9, 41.7, 28.2, 24.7, 22.7, 21.8 ppm. IR (neat): $\tilde{\nu}$ = 3366, 2960, 2873, 1760, 1715, 1514, 1366, 1251, 1167, 1049 cm^{-1}. HRMS (ESI): calcd. for $C_{12}H_{23}O_4NNa$ $[M + Na]^+$ 268.1519; found 268.1527.

Synthesis of 6 and 6a: DIBAL-H (1.0 M in toluene; 166.0 mL, 166.0 mmol) was added to a solution of ester **5** (18.5 g, 75.454 mmol) in toluene at –78 °C under an argon atmosphere. The reaction mixture was stirred for 10 min at that temperature, and then the reaction was quenched by the addition of MeOH (18.5 mL). The reaction mixture was then allowed to warm up to room temp., and a saturated solution of sodium potassium tartrate (85.24 g, 302.040 mmol) was added. The mixture was stirred for 1 h, and then the solids were removed by filtration through a bed of Celite. The mixture was extracted with EtOAc, then the organic phase was washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure to give the aldehyde as a yellow oil. This material was used in the next step without any further purification. R_f = 0.4 (15% EtOAc/hexane).

Isopropylmagnesium bromide (1 M in THF; 151 mL, 150.908 mmol) was added to a solution of aldehyde in THF (150 mL) at 0 °C under a N_2 atmosphere. The reaction was monitored by TLC. The reaction was quenched with water, and the mixture was extracted with EtOAc. The organic extract was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification by column chromatography gave a major product, **6** (12.7 g, 66.2%), as a pale yellow oil, and a minor product, **6a** (3.6 g, 18.7%), as a white solid. R_f = 0.5 (15% EtOAc/hexane).

Data for **6**: $[\alpha]_D^{26}$ = –22.3 (*c* = 0.65, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.68 (br. d, *J* = 9.8 Hz, 1 H), 3.76 (m, 1 H), 3.10 (m, 1 H), 2.16 (br. s, 1 H), 1.83–1.55 (m, 3 H), 1.42 (s, 9 H), 1.35–1.09 (m, 1 H), 1.00–0.86 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.2, 79.3, 78.9, 42.3, 30.9, 28.3, 24.7, 23.0, 22.2, 19.2, 18.3 ppm. IR (neat): $\tilde{\nu}$ = 3440, 2959, 2872, 1688, 1392, 1366, 1250, 1171, 1047 cm^{-1}. HRMS (ESI): calcd. for $C_{14}H_{30}O_3N$ $[M + H]^+$ 260.2220; found 260.2230.

Data for **6a**: m.p. 78–80 °C. $[\alpha]_D^{26}$ = –36.4 (*c* = 0.38, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.66 (br. d, *J* = 9.0 Hz, 1 H), 3.75 (m, 1 H), 3.23 (m, 1 H), 1.90 (br. s, 1 H), 1.73–1.55 (m, 2 H), 1.43 (s, 9 H), 1.37–1.11 (m, 2 H), 1.03–0.87 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.7, 80.3, 79.0, 50.6, 36.9, 30.8, 28.3, 24.5, 23.9, 21.4, 19.2, 18.7 ppm. IR (neat): $\tilde{\nu}$ = 3369, 2956, 1683, 1531, 1273, 1172 cm^{-1}. HRMS (ESI): calcd. for $C_{14}H_{30}O_3N$ $[M + H]^+$ 260.2220; found 260.2234.

***tert*-Butyl [(3*R*,4*S*)-3-Azido-2,6-dimethylheptan-4-yl]carbamate (7):** Et_3N (9.57 mL, 68.793 mmol) and methanesulfonyl chloride (2.0 mL, 25.797 mmol) were added to a solution of alcohol **6** (4.42 g, 17.198 mmol) in CH_2Cl_2 (50 mL) under N_2 at 0 °C. The reaction was monitored by TLC. The reaction mixture was poured into EtOAc, and the mixture was washed with water and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash column chromatography gave the mesylate of **6** (4.5 g, 78%) as a yellow oil. R_f = 0.5 (15% EtOAc/hexane). $[\alpha]_D^{26}$ = –31.9 (*c* = 0.54, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.48 (br. d, *J* = 10.5 Hz, 1 H), 4.35 (dd, *J* = 7.5, 3.0 Hz, 1 H), 4.05–3.93 (m, 1 H), 3.03 (s, 3 H), 2.00 (m, 1 H), 1.74–1.56 (m, 1 H), 1.46 (m, 1 H), 1.42 (s, 9 H), 1.39–1.20 (m, 1 H), 1.07–0.99 (m, 6 H), 0.95 (dd, *J* = 6.0, 2.2 Hz, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.3, 90.4, 79.2, 48.9, 38.6, 29.8, 28.2, 24.4, 22.9, 21.8, 19.2, 17.9 ppm. IR (neat): $\tilde{\nu}$ = 3386, 2963, 2929, 1708, 1511, 1340, 1170, 911 cm^{-1}. HRMS (ESI): calcd. for $C_{15}H_{32}O_5NS$ $[M + H]^+$ 338.1996; found 338.1991.

The mesylate of **6** (4.5 g, 13.392 mmol) was dissolved in dry DMF (20 mL), and sodium azide (1.74 g, 26.785 mmol) was added. The mixture was stirred at 80 °C for 12 h. The reaction mixture was cooled and poured in to ice-water. The mixture was extracted with EtOAc, and the organic extract was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash column chromatography gave azide **7** (3.16 g, 83%) as a white solid. R_f = 0.5 (5% EtOAc/hexane), m.p. 85–87 °C. $[\alpha]_D^{26}$ = –22.3 (*c* = 0.65, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.39 (br. d, *J* = 10.5 Hz, 1 H), 3.95 (m, 1 H), 2.93 (dd, *J* = 9.0, 2.2 Hz, 1 H), 1.80 (m, 1 H), 1.64 (m, 1 H), 1.41 (s, 9 H), 1.36–1.19 (m, 2 H), 1.10–1.00 (m, 6 H), 0.99–0.92 (m, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.2, 79.4, 74.1, 50.1, 37.2, 30.3, 28.3, 24.4, 23.8, 21.2, 20.2, 19.0 ppm. IR (neat): $\tilde{\nu}$ = 3371, 2959, 2925, 2857, 2101, 1681, 1367, 1255, 1169 cm^{-1}. HRMS (ESI): calcd. for $C_{14}H_{28}O_2N_4Na$ $[M + Na]^+$ 307.2104; found 307.2100.

***tert*-Butyl [(3*R*,4*S*)-3-Amino-2,6-dimethylheptan-4-yl]carbamate (3):** A solution of azide **7** (3.16 g, 11.126 mmol) in EtOAc/MeOH (2:1; 30 mL) was hydrogenated at balloon pressure (1 atm) in the presence of a catalytic amount of 10% Pd/C (450 mg). The reaction was monitored by TLC. The mixture was then filtered through a pad of Celite, which was then washed with EtOAc. The solvent was evaporated from the filtrate, and the residue was purified by flash chromatography to give amine **3** (2.64 g, 92%) as a colourless liquid. R_f = 0.4 (EtOAc). $[\alpha]_D^{26}$ = –34.9 (*c* = 1.18, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.90 (br. d, *J* = 9.8 Hz, 1 H), 3.83–3.66 (m, 1 H), 2.27 (dd, *J* = 9.0, 4.5 Hz, 1 H), 1.73–1.58 (m, 1 H), 1.41 (s, 9 H), 1.26–1.03 (m, 3 H), 0.98–0.87 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.5, 78.4, 61.3, 49.8, 37.3, 31.7, 28.3, 24.5, 24.0, 21.4, 19.5 ppm. IR (neat): $\tilde{\nu}$ = 3343, 2961, 2926, 1706, 1368,

1251, 1170 cm^{-1}. HRMS (ESI): calcd. for $C_{14}H_{31}O_2N_2$ [M + H]$^+$ 259.2380; found 259.2390.

***tert*-Butyl {(3*R*,4*S*)-3-[(*S*)-2-Hydroxy-4-methylpentanamido]-2,6-dimethylheptan-4-yl}carbamate (8):** EDCI [1-ethyl-3-(dimethylaminopropyl)carbodiimide] (3.92 g, 20.465 mmol) and HOBt (1-hydroxybenzotriazole; 2.762 g, 20.465 mmol) were added to a stirred solution of acid (–)-**4** (2.01 g, 15.348 mmol) in CH_2Cl_2 (20 mL) at 0 °C, and the reaction mixture was stirred for 10 min. Amine **3** (2.64 g, 10.235 mmol) in CH_2Cl_2 (11 mL) was added to this reaction mixture, followed by DIPEA (8.91 mL, 51.162 mmol). Then the reaction mixture was allowed to stir overnight. The reaction mixture was diluted with EtOAc, and the mixture was washed with HCl (1 N), saturated aq. $NaHCO_3$, and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to give peptide **8** (2.67 g, 70%) as a white solid. R_f = 0.5 (30% EtOAc/hexane), m.p. 124–126 °C. $[\alpha]_D^{25.7}$ = –45 (*c* = 0.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.55 (br. d, *J* = 9.2 Hz, 1 H), 4.55 (br. d, *J* = 8.8 Hz, 1 H), 4.16–4.05 (m, 1 H), 3.81–3.65 (m, 2 H), 3.27 (br. s, 1 H), 1.94–1.76 (m, 2 H), 1.73–1.50 (m, 1 H), 1.43 (s, 9 H), 1.34–1.12 (m, 4 H), 1.02–0.83 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.7, 155.7, 79.2, 70.8, 58.1, 50.2, 44.1, 40.4, 28.9, 28.3, 24.6, 24.5, 23.8, 23.5, 21.5, 21.4, 20.3, 17.8 ppm. IR (neat): $\tilde{\nu}$ = 3447, 2922, 2852, 1654, 1369, 1170 cm^{-1}. HRMS (ESI): calcd. for $C_{20}H_{41}O_4N_2$ [M + H]$^+$ 373.3060; found 373.3078.

(*S*)-1-[(3*R*,4*S*)-4-(*tert*-Butoxycarbonylamino)-2,6-dimethylheptan-3-ylamino]-4-methyl-1-oxopentan-2-yl Methanesulfonate (9): Et_3N (3.99 mL, 28.709 mmol) and methanesulfonyl chloride (0.83 mL, 10.766 mmol) were added to a solution of alcohol **8** (2.67 g, 7.177 mmol) in CH_2Cl_2 (22 mL) under N_2 at 0 °C. The reaction was monitored by TLC. The reaction was quenched with water, and the mixture was extracted with EtOAc. The organic extract was washed with brine, dried with Na_2SO_4, filtered, and concentrated under vacuum. Purification by flash column chromatography gave mesylate **9** (3.13 g, 97%) as a white solid. R_f = 0.5 (30% EtOAc/hexane), m.p. 162–164 °C. $[\alpha]_D^{26}$ = –61.4 (*c* = 0.24, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.30 (br. d, *J* = 9.0 Hz, 1 H), 4.95 (dd, *J* = 9.0, 3.7 Hz, 1 H), 4.48 (br. d, *J* = 8.6 Hz, 1 H), 3.86–3.72 (m, 2 H), 3.11 (s, 3 H), 1.89–1.62 (m, 4 H), 1.44 (s, 9 H), 1.31–1.14 (m, 3 H), 1.05–0.88 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.8, 155.6, 79.1, 78.8, 58.3, 50.3, 41.2, 39.8, 38.7, 28.8, 28.2, 24.6, 24.1, 23.6, 22.9, 21.5, 21.3, 20.0, 17.9 ppm. IR (neat): $\tilde{\nu}$ = 3208, 2956, 2870, 1663, 1468 cm^{-1}. HRMS (ESI): calcd. for $C_{21}H_{43}O_6N_2S$ [M + H]$^+$ 451.2836; found 451.2855.

(3*R*,5*S*,6*R*)-3,5-Diisobutyl-6-isopropylpiperazin-2-one (1): TFA (1.75 mL) was added slowly in a dropwise manner to a solution of **9** (1.1 g, 2.444 mmol) in CH_2Cl_2 (7 mL) under N_2 at 0 °C. The reaction was monitored by TLC. After the reaction was complete, the solvent was evaporated under vacuum to give the amine, which was used in the next reaction without further purification.

The amine was dissolved in dry MeOH (49 mL), and diisopropylethylamine (DIPEA; 1.27 mL, 7.333 mmol) was added. The reaction mixture was heated at reflux overnight. After the reaction was complete, the solvent was evaporated under reduced pressure, and the residue was purified by column chromatography to give cyclic compound **1** (509 mg, 82%) as a white solid. R_f = 0.5 (30% EtOAc/hexanes), m.p. 53–55 °C. $[\alpha]_D^{26}$ = +52.5 (*c* = 0.33, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.21 (br. s, 1 H), 3.38 (dd, *J* = 9.8, 3.7 Hz, 1 H), 3.15 (m, 1 H), 3.07 (dd, *J* = 6.7, 3.7 Hz, 1 H), 1.99–1.82 (m, 2 H), 1.81–1.59 (m, 1 H), 1.44–1.22 (m, 4 H), 1.02–0.80 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.2, 59.3, 56.8, 53.3, 41.1, 40.4, 27.8, 24.8, 24.4, 23.6, 23.0, 22.4, 21.5, 20.9, 17.7 ppm. IR (neat): $\tilde{\nu}$ = 3208, 2956, 1663, 1468, 1165, 1117 cm^{-1}. HRMS (ESI): calcd. for $C_{15}H_{30}ON_2$ [M + Na]$^+$ 277.2255; found 277.2266.

***tert*-Butyl [(3*S*,4*S*)-3-Azido-2,6-dimethylheptan-4-yl]carbamate (7a):** The mesylate of **6a** (994 g, 78%) was prepared as a yellow oil from **6a** (975 mg, 3.793 mmol) following the procedure described for the synthesis of the mesylate of **6**. R_f = 0.5 (15% EtOAc/hexane). $[\alpha]_D^{26}$ = –7.1 (*c* = 0.91, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.83 (br. d, *J* = 8.0 Hz, 1 H), 4.54 (br. d, *J* = 8.0 Hz, 1 H), 3.93 (m, 1 H), 3.04 (s, 3 H), 1.90 (m, 1 H), 1.67 (m, 1 H), 1.42 (s, 9 H), 1.36 (m, 1 H), 1.23 (m, 1 H), 1.07–0.83 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.4, 91.3, 79.3, 49.3, 38.4, 37.0, 29.8, 28.3, 24.3, 23.7, 21.2, 19.5, 18.7 ppm. IR (neat): $\tilde{\nu}$ = 3388, 2964, 2927, 1708, 1513, 1339, 1170, 912 cm^{-1}. HRMS (ESI): calcd. for $C_{15}H_{32}O_5NS$ [M + H]$^+$ 338.1996; found 338.1998.

Azide **7a** (647 mg, 77%) was prepared as a colourless liquid from the mesylate of **6a** (994 mg, 2.958 mmol) following the procedure described for the synthesis of **7**. R_f = 0.5 (5% EtOAc/hexane). $[\alpha]_D^{26}$ = –45.2 (*c* = 0.37, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.40 (br. d, *J* = 9.8 Hz, 1 H), 3.94 (m, 1 H), 2.93 (dd, *J* = 9.0, 2.2 Hz, 1 H), 1.80 (m, 1 H), 1.63 (m, 1 H), 1.41 (s, 9 H), 1.33–1.18 (m, 2 H), 1.08–0.99 (m, 6 H), 0.98–0.92 (m, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.4, 79.1, 74.0, 49.3, 43.6, 30.8, 28.3, 24.8, 22.9, 22.3, 19.7, 19.6 ppm. IR (neat): $\tilde{\nu}$ = 3392, 2961, 2925, 2099, 1646, 1166 cm^{-1}. HRMS (ESI): calcd. for $C_{14}H_{28}O_2N_4Na$ [M + Na]$^+$ 307.2104; found 307.2100.

***tert*-Butyl [(3*S*,4*S*)-3-Amino-2,6-dimethylheptan-4-yl]carbamate (3a):** Compound **3a** (547 mg, 93%) was prepared as a colourless liquid from azide **7a** (647 mg, 2.278 mmol) following the procedure described for the synthesis of **3**. R_f = 0.4 (EtOAc). $[\alpha]_D^{26}$ = –33.3 (*c* = 0.48, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.81 (br. d, *J* = 9.0 Hz, 1 H), 3.75 (m, 1 H), 2.33 (m, 1 H), 1.70–1.50 (m, 2 H), 1.41 (s, 9 H), 1.33 (m, 1 H), 1.14 (m, 1 H), 0.98–0.88 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.8, 78.4, 59.8, 49.4, 43.2, 30.6, 28.2, 24.7, 22.9, 22.2, 19.8, 18.6 ppm. IR (neat): $\tilde{\nu}$ = 3362, 2960, 1646, 1250, 1171 cm^{-1}. HRMS (ESI): calcd. for $C_{14}H_{31}O_2N_2$ [M + H]$^+$ 259.2380; found 259.2395.

***tert*-Butyl [(3*S*,4*S*)-3-[(*S*)-2-Hydroxy-4-methylpentanamido]-2,6-dimethylheptan-4-yl]carbamate (8a):** Compound **8a** (513 mg, 65%) was prepared as a white solid from the coupling of acid (–)-**4** (417 mg, 3.180 mmol) and amine **3a** (547 mg, 2.120 mmol) following the procedure described for the synthesis of **8**. R_f = 0.5 (30% EtOAc/hexane), m.p. 140–142 °C. $[\alpha]_D^{26}$ = –85.2 (*c* = 0.085, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.76 (br. d, *J* = 9.8 Hz, 1 H), 5.78 (br. d, *J* = 9.0 Hz, 0.4 H), 4.68 (br. d, *J* = 8.3 Hz, 0.6 H), 4.12 (m, 1 H), 3.80–3.48 (m, 2 H), 1.96–1.79 (m, 2 H), 1.73–1.49 (m, 2 H), 1.40 (s, 9 H), 1.31–1.14 (m, 4 H), 0.99–0.78 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.2, 156.8, 79.3, 71.0, 58.3, 57.9, 50.6, 43.6, 41.5, 28.7, 28.3, 24.5, 23.5, 21.5, 21.2 ppm. IR (neat): $\tilde{\nu}$ = 3360, 2957, 2922, 2853, 1741, 1647, 1250, 1171, 1091, 1044 cm^{-1}. HRMS (ESI): calcd. for $C_{20}H_{41}O_4N_2$ [M + H]$^+$ 373.3060; found 373.3080.

(*S*)-1-[(3*S*,4*S*)-4-(*tert*-Butoxycarbonylamino)-2,6-dimethylheptan-3-ylamino]-4-methyl-1-oxopentan-2-yl Methanesulfonate (9a): Mesylate **9a** (485 mg, 78%) was prepared as a colourless liquid from **8a** (513 g, 1.379 mmol) following the procedure described for the synthesis of **9**. R_f = 0.5 (30% EtOAc/hexane). $[\alpha]_D^{26}$ = –89.8 (*c* = 0.57, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.49 (br. d, *J* = 7.9 Hz, 1 H), 5.09–5.03 (m, 1 H), 4.71 (br. d, *J* = 8.9 Hz, 1 H), 3.90 (br. s, 1 H), 3.62 (m, 1 H), 3.15 (s, 3 H), 1.84–1.71 (m, 4 H), 1.67–1.58 (m, 1 H), 1.43 (s, 9 H), 1.37–1.28 (m, 2 H), 1.08–0.86 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.8, 156.1, 79.0, 58.3, 50.2, 49.7, 42.1, 41.3, 38.7, 29.7, 28.3, 24.6, 24.4, 23.1, 22.9, 22.0,

20.9, 20.1, 18.8 ppm. IR (neat): ṽ = 3389, 2958, 2923, 2855, 1677, 1646, 1360, 1171, 1092 cm^{-1}. HRMS (ESI): calcd. for $C_{21}H_{42}O_6N_2NaS$ [M + Na]$^+$ 473.2655; found 473.2655.

(3*R*,5*S*,6*S*)-3,5-Diisobutyl-6-isopropylpiperazin-2-one (1a): Cyclic compound **1a** (84 mg, 74%) was prepared as an amorphous solid from **9a** (200 mg, 0.444 mmol) following the procedure described for the synthesis of **1**. R_f = 0.4 (50% EtOAc/hexane). $[\alpha]_D^{26}$ = –61.5 (c = 0.065, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.72 (br. s, 1 H), 3.43 (dd, J = 10.3, 3.9 Hz, 1 H), 2.93–2.84 (m, 2 H), 1.99–1.73 (m, 2 H), 1.71–1.48 (m, 4 H), 1.35–1.27 (m, 1 H), 0.99–0.85 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.6, 63.4, 53.5, 46.8, 41.1, 40.6, 29.5, 24.5, 24.4, 23.6, 23.5, 21.5, 21.0, 19.7, 16.0 ppm. IR (neat): ṽ = 3200, 2956, 2927, 1693, 1315 cm^{-1}. HRMS (ESI): calcd. for $C_{15}H_{31}ON_2$ [M + H]$^+$ 255.2430; found 255.2433.

***tert*-Butyl [(3*R*,4*S*)-3-[(*R*)-2-Hydroxy-4-methylpentanamido]-2,6-dimethylheptan-4-yl]carbamate (8b):** Compound **8b** (1.0 g, 52%) was prepared as a white solid from the coupling of acid (+)-**4** (1.0 g, 7.674 mmol) and amine **3** (1.32 g, 5.116 mmol) following the procedure described for the synthesis of **8**. R_f = 0.5 (30% EtOAc/hexane), m.p. 58–60 °C. $[\alpha]_D^{26}$ = –35.6 (c = 0.07, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.53 (br. d, J = 10.5 Hz, 0.5 H), 6.06 (br. d, J = 8.3 Hz, 0.5 H), 4.57 (m, 1 H), 4.18–4.06 (m, 1 H), 3.86–3.66 (m, 2 H), 1.96–1.56 (m, 3 H), 1.45 (m, 9 H), 1.32–1.05 (m, 4 H), 1.01–0.83 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.8, 155.9, 79.3, 71.1, 57.9, 50.5, 43.9, 40.4, 28.9, 28.3, 24.7, 24.6, 23.7, 23.4, 21.6, 21.3, 20.4 ppm. IR (neat): ṽ = 3391, 2957, 2922, 2853, 1705, 1647, 1367, 1171, 1079 cm^{-1}. HRMS (ESI): calcd. for $C_{20}H_{41}O_4N_2$ [M + H]$^+$ 373.3060; found 373.3068.

(*R*)-1-[(3*R*,4*S*)-4-(*tert*-Butoxycarbonylamino)-2,6-dimethylheptan-3-ylamino]-4-methyl-1-oxopentan-2-yl Methanesulfonate (9b): Mesylate **9b** (992 mg, 82%) was prepared as a white solid from **8b** (1.0 g, 2.688 mmol) following the procedure described for the synthesis of **9**. R_f = 0.5 (30% EtOAc/hexane), m.p. 144–146 °C. $[\alpha]_D^{26}$ = –5.0 (c = 0.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.31 (br. d, J = 9.8 Hz, 1 H), 5.00 (m, 1 H), 4.61 (br. d, J = 9.0 Hz, 1 H), 3.91–3.77 (m, 2 H), 3.13 (s, 3 H), 1.90–1.56 (m, 5 H), 1.42 (s, 9 H), 1.30–1.13 (m, 2 H), 1.05–0.85 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.9, 155.8, 79.3, 58.4, 50.0, 41.5, 38.8, 38.3, 29.4, 28.3, 24.6, 24.4, 23.7, 23.1, 21.4, 21.1, 19.9, 18.7 ppm. IR (neat): ṽ = 3366, 2957, 2922, 2853, 1741, 1648, 1395, 1177 cm^{-1}. HRMS (ESI): calcd. for $C_{21}H_{42}O_6N_2NaS$ [M + H]$^+$ 473.2655; found 473.2658.

(3*S*,5*S*,6*R*)-3,5-Diisobutyl-6-isopropylpiperazin-2-one (1b): Cyclic compound **1b** (23 mg, 64%) was prepared as a colourless liquid from **9b** (100 mg, 0.222 mmol) following the procedure described for the synthesis of **1**. R_f = 0.5 (30% EtOAc/hexane). $[\alpha]_D^{26}$ = –73.5 (c = 0.06, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.23 (br. s, 1 H), 3.43–3.34 (m, 2 H), 2.57 (dd, 10.4, 3.1, 1 H), 1.87 (m, 1 H), 1.79 (m, 1 H), 1.69 (m, 1 H), 1.61 (m, 1 H), 1.46 (m, 1 H), 1.29 (m, 1 H), 1.11 (m, 1 H), 1.03–0.83 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.6, 62.6, 57.6, 51.5, 41.0, 38.4, 28.5, 24.4, 24.2, 23.8, 23.5, 20.9, 20.8, 20.2, 19.0 ppm. IR (neat): ṽ = 3207, 2956, 2926, 1693, 1315 cm^{-1}. HRMS (ESI): calcd. for $C_{15}H_{31}ON_2$ [M + H]$^+$ 255.2430; found 255.2432.

***tert*-Butyl [(3*S*,4*S*)-3-[(*R*)-2-Hydroxy-4-methylpentanamido]-2,6-dimethylheptan-4-yl]carbamate (8c):** Compound **8c** (382 mg, 53%) was prepared as a white solid from the coupling of acid (+)-**4** (381 mg, 2.906 mmol) and amine **3a** (500 mg, 1.937 mmol) following the procedure described for the synthesis of **8**. R_f = 0.5 (30% EtOAc/hexane), m.p. 108–110 °C. $[\alpha]_D^{26}$ = –40 (c = 0.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.60 (br. d, J = 9.8 Hz, 1 H), 4.54 (br. d, J = 8.3 Hz, 1 H), 4.12 (m, 1 H), 3.79–3.58 (m, 2 H), 3.01 (br. s, 1 H), 1.95–1.73 (m, 4 H), 1.71–1.48 (m, 1 H), 1.41 (s, 9 H), 1.33–1.22 (m, 2 H), 1.01–0.84 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.5, 156.6, 79.2, 70.5, 58.1, 50.1, 44.2, 42.0, 28.9, 28.3, 24.7, 24.4, 23.5, 21.5, 21.1, 20.4 ppm. IR (neat): ṽ = 3332, 2957, 2922, 2855, 1740, 1690, 1367, 1170, 1091 cm^{-1}. HRMS (ESI): calcd. for $C_{20}H_{41}O_4N_2$ [M + H]$^+$ 373.3060; found 373.3059.

(*R*)-1-[(3*S*,4*S*)-4-(*tert*-Butoxycarbonylamino)-2,6-dimethylheptan-3-ylamino]-4-methyl-1-oxopentan-2-yl Methanesulfonate (9c): Mesylate **9c** (333 mg, 72%) was prepared as a white solid from **8c** (382 mg, 1.027 mmol) following the procedure described for the synthesis of **9**. R_f = 0.5 (30% EtOAc/hexane), m.p. 94–96 °C. $[\alpha]_D^{26}$ = +2.26 (c = 0.77, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.74 (br. d, J = 8.0 Hz, 1 H), 5.04 (m, 1 H), 4.51 (br. d, J = 7.0 Hz, 1 H), 3.76–3.63 (m, 2 H), 3.13 (s, 3 H), 1.94–1.60 (m, 5 H), 1.42 (s, 9 H), 1.35–1.27 (m, 2 H), 1.04–0.86 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.4, 156.4, 78.2, 58.4, 50.2, 41.8, 41.4, 38.6, 29.2, 28.3, 24.7, 24.2, 23.3, 23.1, 21.6, 21.0, 20.2 ppm. IR (neat): ṽ = 3360, 2959, 2925, 2860, 1741, 1684, 1362, 1248, 1172 cm^{-1}. HRMS (ESI): calcd. for $C_{21}H_{43}O_6N_2S$ [M + H]$^+$ 451.2836; found 451.2834.

(3*S*,5*S*,6*S*)-3,5-Diisobutyl-6-isopropylpiperazin-2-one (1c): Cyclic compound **1c** (60 mg, 71%) was prepared as a white solid from **9c** (150 mg, 0.333 mmol) following the procedure described for the synthesis of **1**. R_f = 0.4 (50% EtOAc/hexane), m.p. 74–76 °C. $[\alpha]_D^{26}$ = –59.8 (c = 0.29, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.01 (br. s, 1 H), 3.41 (dd, J = 10.1, 4.1 Hz, 1 H), 2.94–2.82 (m, 2 H), 1.98–1.72 (m, 3 H), 1.69–1.47 (m, 2 H), 1.36–1.21 (m, 2 H), 1.01–0.85 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.5, 63.2, 53.3, 46.7, 41.0, 40.5, 29.5, 24.4, 24.3, 23.5, 23.4, 21.5, 20.9, 19.6, 16.0 ppm. IR (neat): ṽ = 3207, 2954, 2929, 1649, 1667 cm^{-1}. HRMS (ESI): calcd. for $C_{15}H_{31}ON_2$ [M + H]$^+$ 255.2430; found 255.2435.

Methyl (*S*)-2-(*tert*-Butoxycarbonylamino)-3-methylbutanoate (10):[26] Compound **10** (9.1 g, 98%) was prepared as a colourless oil from L-valine (5 g, 42.680 mmol) following the procedure described for the synthesis of **5**. R_f = 0.5 (15% EtOAc/hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.01 (br. d, J = 9.0 Hz, 1 H), 4.22 (dd, J = 9.0, 4.5 Hz, 1 H), 3.73 (s, 3 H), 2.11 (m, 1 H), 1.44 (s, 9 H), 0.95 (d, J = 6.7 Hz, 3 H), 0.88 (d, J = 6.7 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.7, 155.5, 79.5, 58.4, 51.8, 31.1, 28.1, 18.8, 17.5 ppm. IR (neat): ṽ = 3342, 2961, 2921, 1760, 1714, 1503, 1243, 1170 cm^{-1}. HRMS (ESI): calcd. for $C_{11}H_{21}O_4NNa$ [M + Na]$^+$ 254.1367; found 254.1369.

***tert*-Butyl [(3*S*)-4-Hydroxy-2,6-dimethylheptan-3-yl]carbamate (11):** A diastereomeric mixture of alcohols **11** (dr = 7:1; 8.68 g, 80%) was prepared as a yellow oil by the reaction of isobutylmagnesium bromide (1 M in THF; 83.8 mL, 83.8 mmol) with the aldehyde derived from **10** (9.1 g, 41.900 mmol) following the procedure described for the synthesis of **6** and **6a**. R_f = 0.5 (15% EtOAc/hexane). $[\alpha]_D^{26}$ = +40.2 (c = 0.23, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.71 (br. d, J = 9.8 Hz, 1 H), 3.83 (m, 1 H), 3.11 (m, 1 H), 1.90–1.68 (m, 2 H), 1.43 (s, 9 H), 1.36–1.21 (m, 2 H), 1.02–0.86 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.7, 78.8, 68.9, 59.9, 43.8, 30.0, 28.3, 24.3, 23.1, 22.2, 19.9, 19.1 ppm. IR (neat): ṽ = 3394, 2957, 2922, 2854, 1689, 1245, 1169 cm^{-1}. HRMS (ESI): calcd. for $C_{14}H_{30}O_3N$ [M + H]$^+$ 260.2220; found 260.2228.

Benzyl [(2*S*)-1-[(3*S*)-4-Hydroxy-2,6-dimethylheptan-3-ylamino]-4-methyl-1-oxopentan-2-yl]carbamate (13): TFA (16 mL) was added slowly in a dropwise manner to a solution of compound **11** (5.62 g, 21.698 mmol) in CH_2Cl_2 (65 mL) under N_2 at 0 °C. The reaction was monitored by TLC. After the reaction was complete, the solvent was evaporated to give the free amine.

Compound **12** (Cbz-L-Leu-OH)[27] (740 mg, 2.825 mmol) was dissolved in CH_2Cl_2 (2 mL), and EDCI (722.16 mg, 3.767 mmol) and HOBt (508 mg, 3.767 mmol) were added at 0 °C. The reaction mixture was stirred for 10 min. Then the free amine (300 mg, 1.883 mmol) prepared above was added in CH_2Cl_2 (4 mL), and then DIPEA (1.64 mL, 9.415 mmol) was added. The reaction mixture was allowed to stir overnight. The reaction mixture was diluted with EtOAc, and this mixture was washed with HCl (1 N), saturated aq. $NaHCO_3$, and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to give peptide **13** (512 mg, 67%). $[a]_D^{26}$ = –22.7 (*c* = 0.33, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.33–7.22 (m, 5 H), 6.61 (br. d, *J* = 8.4 Hz, 1 H), 5.43 (br. d, *J* = 8.4 Hz, 1 H), 5.12–5.00 (m, 2 H), 4.19–4.10 (m, 1 H), 3.88 (m, 1 H), 3.45 (m, 1 H), 1.89–1.78 (m, 1 H), 1.75–1.58 (m, 4 H), 1.48 (m, 1 H), 1.17 (m, 1 H), 1.00–0.80 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.4, 156.2, 136.1, 128.4, 128.0, 127.9, 68.3, 66.9, 58.6, 53.9, 43.9, 41.2, 29.7, 24.6, 24.3, 23.0, 22.6, 22.2, 19.7, 19.2 ppm. IR (neat): $\tilde{\nu}$ = 3315, 2956, 2929, 2871, 1694, 1647, 1531, 1462, 1257, 1120, 1042 cm^{-1}. HRMS (ESI): calcd. for $C_{23}H_{39}O_4N_2$ $[M + H]^+$ 407.2904; found 407.2920.

Benzyl [(*S*)-1-[(*S*)-2,6-Dimethyl-4-oxoheptan-3-ylamino]-4-methyl-1-oxopentan-2-yl]carbamate (14): Dess–Martin periodinane (DMP; 641.6 mg, 1.513 mmol) was added to a stirred solution of alcohol **13** (512 mg, 1.261 mmol) in CH_2Cl_2 (4 mL) at 0 °C. The resulting solution was stirred at room temp., and the reaction was monitored by TLC. The reaction was quenched with a mixture of saturated aq. $NaHCO_3$ and saturated aq. $Na_2S_2O_3$ (1:1). The resulting mixture was stirred for 15 min to give a clear solution. The aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography to give ketone **14** (450 mg, 88%) as a white solid. R_f = 0.5 (30% EtOAc/hexane), m.p. 80–82 °C. $[a]_D^{26}$ = +22.1 (*c* = 0.52, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.34–7.24 (m, 5 H), 6.52 (br. d, *J* = 7.8 Hz, 1 H), 5.20 (br. d, *J* = 7.8 Hz, 1 H), 5.12–5.04 (m, 2 H), 4.51 (dd, *J* = 8.7, 3.9 Hz, 1 H), 4.18 (m, 1 H), 2.44–2.27 (m, 2 H), 2.23–2.10 (m, 2 H), 1.71–1.57 (m, 2 H), 1.49 (m, 1 H), 0.98–0.87 (m, 16 H), 0.79–0.72 (m, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 208.4, 172.5, 156.0, 136.1, 128.2, 127.8, 127.7, 66.7, 62.4, 53.4, 49.7, 41.2, 29.8, 24.4, 23.9, 22.6, 22.4, 22.2, 21.9, 19.7, 16.6 ppm. IR (neat): $\tilde{\nu}$ = 3304, 2958, 2926, 1706, 1695, 1532, 1462, 1122, 1043 cm^{-1}. HRMS (ESI): calcd. for $C_{23}H_{37}O_4N_2$ $[M + H]^+$ 405.2753; found 405.2761.

(3*S*,5*R*,6*S*)-3,5-Diisobutyl-6-isopropylpiperazin-2-one (–)-1: A solution of ketone **14** (200 mg, 0.495 mmol) in MeOH (4 mL) was hydrogenated at balloon pressure (1 atm) in the presence of 10% Pd/C (200 mg). The reaction was monitored by TLC. The mixture was filtered through a pad of Celite, which was then washed with EtOAc. The solvent was evaporated from the filtrate under reduced pressure, and the residue was purified by column chromatography to give cyclic compound (–)-**1** (94 mg, 74%) as a white solid, m.p. 53–55 °C. $[a]_D^{26}$ = –55.2 (*c* = 0.65, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.66 (br. s, 1 H), 3.36 (dd, *J* = 9.8, 3.7 Hz, 1 H), 3.15 (m, 1 H), 3.06 (dd, *J* = 6.7, 3.7 Hz, 1 H), 1.95–1.82 (m, 1 H), 1.81–1.60 (m, 1 H), 1.42–1.24 (m, 5 H), 1.04–0.88 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.4, 59.2, 56.8, 53.2, 41.1, 40.4, 27.8, 24.7, 24.3, 23.5, 23.0, 22.4, 21.5, 20.9, 17.7 ppm. IR (neat): $\tilde{\nu}$ = 3343, 2957, 2871, 1663, 1469, 1280, 1164 cm^{-1}. HRMS (ESI): calcd. for $C_{15}H_{31}ON_2$ $[M + H]^+$ 255.2430; found 255.2440.

(3*S*,5*R*,6*S*)-4-Acetyl-3,5-diisobutyl-6-isopropylpiperazin-2-one (15): Ac_2O (0.16 mL, 0.1338 mmol), Et_3N (0.022 mL, 0.1606 mmol), and DMAP (catalytic amount) were added to a solution of compound (–)-**1** (34 mg, 0.1338 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C, and the reaction mixture was allowed to stir for 30 min. After the reaction was complete, H_2O was added, and the mixture was stirred for a further 10 min. The mixture was extracted with EtOAc, and the organic phase was washed with water and brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to give acetylated compound **15** (31 mg, 79%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.96 (br. s, 1 H), 5.17 (d, *J* = 13.2 Hz, 0.4 H), 5.03 (t, *J* = 6.6 Hz, 0.6 H), 4.22 (dd, *J* = 8.8, 4.4 Hz, 0.4 H), 3.98 (d, *J* = 12.1 Hz, 0.6 H), 3.06 (dd, *J* = 11.0, 3.3 Hz, 0.6 H), 3.00 (dd, *J* = 9.9, 3.3 Hz, 0.4 H), 2.26 (m, 0.5 H), 2.15 (s, 3 H), 2.04 (m, 0.5 H), 1.92 (m, 1 H), 1.70–1.54 (m, 3 H), 1.34 (m, 1 H), 1.10 (m, 1 H), 1.05–0.93 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.7, 170.5, 169.9, 169.5, 62.6, 61.5, 54.8, 52.8, 52.0, 46.4, 45.5, 43.9, 33.7, 29.2, 29.1, 25.7, 25.5, 23.8, 23.6, 23.5, 23.4, 23.2, 22.5, 22.3, 22.2, 22.0, 21.7, 21.5, 21.4, 19.5, 18.0, 17.9 ppm. IR (neat): $\tilde{\nu}$ = 3441, 2925, 2858, 1667, 1631, 1325, 1115 cm^{-1}. HRMS (ESI): calcd. for $C_{17}H_{33}O_2N_2$ $[M + H]^+$ 297.2536; found 297.2547.

Benzyl [2-[(3*S*)-4-Hydroxy-2,6-dimethylheptan-3-ylamino]-2-oxoethyl]carbamate (16a): EDCI (192.7 mg, 1.006 mmol) and HOBt (135.8 mg, 1.006 mmol) were added to a stirred solution of acid **12a** (Cbz-Gly-OH; 157.7 mg, 0.7547 mmol) in CH_2Cl_2 (2 mL) at 0 °C, and the reaction mixture was stirred for 10 min. The free amine derived from **11** (TFA, CH_2Cl_2; 80 mg, 0.503 mmol) was added in CH_2Cl_2 (3 mL), followed by DIPEA (0.43 mL, 2.5157 mmol). The reaction mixture was allowed to stir overnight. The reaction mixture was diluted with EtOAc, and this mixture was washed with HCl (1 N), saturated aq. $NaHCO_3$, and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to give peptide **16a** (113 mg, 64%). R_f = 0.4 (60% EtOAc/hexane). $[a]_D^{26}$ = –14.5 (*c* = 0.84, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.42–7.25 (m, 5 H), 6.13 (br. d, *J* = 9.6 Hz, 1 H), 5.76 (br. s, 1 H), 5.48 (br. s, 1 H), 5.20–5.01 (m, 3 H), 4.00–3.72 (m, 2 H), 1.72 (m, 1 H), 1.55 (m, 1 H), 1.42–1.22 (m, 2 H), 0.98–0.77 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.2, 169.6, 156.7, 136.0, 128.4, 128.0, 127.9, 73.0, 67.1, 67.0, 56.8, 44.6, 42.6, 40.5, 29.1, 24.3, 23.0, 21.7, 19.9 ppm. IR (neat): $\tilde{\nu}$ = 3333, 2958, 2924, 2855, 1707, 1676, 1518, 1461, 1392, 1265, 1199, 1052 cm^{-1}. HRMS (ESI): calcd. for $C_{19}H_{31}O_4N_2$ $[M + H]^+$ 351.2283; found 351.2274.

Benzyl [(2*S*)-1-[(3*S*)-4-Hydroxy-2,6-dimethylheptan-3-ylamino]-1-oxopropan-2-yl]carbamate (16b): Compound **16b** (125 mg, 54%) was prepared as a colourless oil from the coupling of **12b** (Cbz-L-Ala-OH)[29] (210 mg, 0.9433 mmol) and the free amine derived from **11** (TFA, CH_2Cl_2; 100 mg, 0.628 mmol) following the procedure described for the synthesis of **16a**. R_f = 0.4 (50% EtOAc/hexane). $[a]_D^{26}$ = –65.3 (*c* = 0.13, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.30 (m, 5 H), 6.35 (br. d, *J* = 8.8 Hz, 1 H), 5.48 (br. d, *J* = 7.1 Hz, 1 H), 5.18–5.05 (m, 2 H), 4.25 (m, 1 H), 3.90 (m, 1 H), 3.49 (m, 1 H), 1.96–1.60 (m, 3 H), 1.51–1.36 (m, 3 H), 1.35–1.11 (m, 1 H), 1.07–0.81 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.4, 155.8, 136.1, 128.5, 128.1, 128.0, 68.6, 66.9, 58.5, 50.8, 43.8, 29.8, 24.4, 23.1, 22.2, 19.7, 19.2 ppm. IR (neat): $\tilde{\nu}$ = 3393, 2957, 2925, 1740, 1694, 1516, 1248, 1049 cm^{-1}. HRMS (ESI): calcd. for $C_{20}H_{33}O_4N_2$ $[M + H]^+$ 365.2440; found 365.2445.

Benzyl [(2*S*)-1-[(3*S*)-4-Hydroxy-2,6-dimethylheptan-3-ylamino]-3-methyl-1-oxobutan-2-yl]carbamate (16c): Compound **16c** (142 mg, 57%) was prepared as a colourless liquid from the coupling of **12c** (Cbz-L-Val-OH; 236.7 mg, 0.9433 mmol) and the free amine de-

rived from **11** (100 mg, 0.6289 mmol) following the procedure described for the synthesis of **16a**. R_f = 0.4 (30% EtOAc/hexane). $[\alpha]_D^{26}$ = –26.6 (*c* = 0.26, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.29 (m, 5 H), 6.28 (br. d, *J* = 8.8 Hz, 1 H), 5.38 (br. d, *J* = 8.6 Hz, 1 H), 5.18–5.04 (dd, *J* = 17.9, 12.2 Hz, 2 H), 3.98 (dd, *J* = 8.8, 6.2 Hz, 1 H), 3.91 (m, 1 H), 3.56–3.47 (m, 1 H), 2.25–1.97 (m, 2 H), 1.86 (m, 1 H), 1.39–1.15 (m, 2 H), 1.04–0.82 (m, 18 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.5, 156.5, 136.2, 128.4, 128.0, 127.8, 68.4, 66.9, 61.0, 58.5, 44.1, 30.5, 29.9, 24.3, 22.9, 22.3, 19.7, 19.4, 17.8 ppm. IR (neat): $\tilde{\nu}$ = 3391, 2925, 1694, 1647, 1514, 1171, 1094 cm^{-1}. HRMS (ESI): calcd. for $C_{22}H_{37}O_4N_2$ $[M + H]^+$ 393.2753; found 393.2761.

Benzyl [(2*S*,3*S*)-1-[(3*S*)-4-Hydroxy-2,6-dimethylheptan-3-ylamino]-3-methyl-1-oxopentan-2-yl]carbamate (16d): Compound **16d** (158 mg, 62%) was prepared as a solid from the coupling of **12d** (Cbz-L-Ileu-OH)[30] (249.9 mg, 0.9433 mmol) with the free amine derived from **11** (100 mg, 0.6289 mmol) following the procedure described for the synthesis of **16a**. R_f = 0.35 (30% EtOAc/hexane), m.p. 110–112 °C. $[\alpha]_D^{26}$ = –40.6(*c* = 0.08, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.38–7.28 (m, 5 H), 6.18 (br. d, *J* = 9.0 Hz, 1 H), 5.33 (br. d, *J* = 9.0 Hz, 1 H), 5.10 (dd, *J* = 19.6, 12.0 Hz, 2 H), 4.01 (m, 1 H), 3.92 (m, 1 H), 3.51 (m, 1 H), 1.98–1.77 (m, 2 H), 1.75–1.60 (m, 2 H), 1.39–1.05 (m, 3 H), 1.02–0.83 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.5, 156.4, 136.2, 128.4, 128.0, 127.9, 68.3, 66.9, 60.1, 58.4, 44.1, 36.7, 29.9, 24.5, 24.3, 22.9, 22.3, 19.8, 19.3, 15.6, 11.1 ppm. IR (neat): $\tilde{\nu}$ = 3421, 2959, 2924, 2854, 1741, 1693, 1516, 1463, 1340, 1315 cm^{-1}. HRMS (ESI): calcd. for $C_{23}H_{39}O_4N_2$ $[M + H]^+$ 407.2909; found 407.2916.

Benzyl [(2*S*)-1-[(3*S*)-4-Hydroxy-2,6-dimethylheptan-3-ylamino]-1-oxo-3-phenylpropan-2-yl]carbamate (16e): Compound **16e** (155 mg, 70%) was prepared as a solid from coupling of **12e** (Cbz-L-Phe-OH; 225 mg, 0.7547 mmol) with the free amine derived from **11** (80 mg, 0.503 mmol) following the procedure described for the synthesis of **16a**. R_f = 0.4 (30% EtOAc/hexane), m.p. 95–97 °C. $[\alpha]_D^{26}$ = –8.2 (*c* = 0.69, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.18 (m, 10 H), 6.06 (br. d, *J* = 9.0 Hz, 1 H), 5.35 (br. s, 1 H), 5.08 (dd, *J* = 19.6, 12.0 Hz, 2 H), 4.43 (m, 1 H), 3.80 (m, 1 H), 3.46 (m, 1 H), 3.16–3.04 (m, 2 H), 1.85–1.56 (m, 2 H), 1.30–1.01 (m, 2 H), 0.98–0.65 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.0, 170.9, 156.0, 155.9, 136.5, 136.3, 129.2, 128.7, 128.6, 128.4, 128.1, 128.0, 127.0, 126.9, 68.8, 68.5, 67.0, 58.7, 58.6, 43.8, 43.7, 38.2, 38.1, 38.0, 29.9, 29.7, 24.4, 24.3, 23.1, 22.1, 22.1, 19.7, 19.0, 18.9 ppm. IR (neat): $\tilde{\nu}$ = 3314, 2956, 2927, 1695, 1650, 1531, 1461, 1393, 1256, 1047 cm^{-1}. HRMS (ESI): calcd. for $C_{26}H_{37}O_4N_2$ $[M + H]^+$ 441.2753; found 441.2753.

Benzyl [2-[(4*S*)-3-Hydroxy-2,6-dimethylheptan-4-ylamino]-2-oxoethyl]carbamate (17a): Compound **17a** (119 mg, 54%) was prepared as a colourless liquid from the coupling of **12a** (Cbz-Gly-OH; 197 mg, 0.9433 mmol) with the free amine derived from **6** and **6a** (TFA, CH_2Cl_2; 100 mg, 0.6289 mmol) following the procedure described for the synthesis of **16a**. R_f = 0.4 (60% EtOAc/hexane). $[\alpha]_D^{26}$ = –9.0 (*c* = 1.02, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.38–7.28 (m, 5 H), 6.22 (br. s, 1 H), 5.60 (br. s, 1 H), 5.11 (s, 2 H), 4.16 (m, 1 H), 3.96 (m, 1 H), 3.84 (m, 1 H), 3.14 (br. d, *J* = 7.9 Hz, 1 H), 1.58 (m, 1 H), 1.46 (m, 1 H), 1.29 (m, 2 H), 0.98–0.86 (m, 12 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 168.8, 156.5, 156.4, 136.0, 128.4, 128.1, 128.0, 127.9, 78.9, 67.0, 62.5, 48.8, 44.4, 42.5, 41.9, 30.9, 24.6, 22.9, 22.2, 19.1, 18.4 ppm. IR (neat): $\tilde{\nu}$ = 3358, 3333, 2956, 2924, 2862, 1707, 1656, 1518, 1461, 1374, 1248, 1173, 1052 cm^{-1}. HRMS (ESI): calcd. for $C_{19}H_{31}O_4N_2$ $[M + H]^+$ 351.2283; found 351.2286.

Benzyl [(2*S*)-1-[(4*S*)-3-Hydroxy-2,6-dimethylheptan-4-ylamino]-1-oxopropan-2-yl]carbamate (17b): Compound **17b** (157 mg, 68%) was prepared as a pale yellow oil from the coupling of **12b** (Cbz-L-Ala-OH; 210 mg, 0.9433 mmol) and the free amine derived from **6** and **6a** (100 mg, 0.6289 mmol) following the procedure described for the synthesis of **16a**. R_f = 0.4 (50% EtOAc/hexane). $[\alpha]_D^{25.7}$ = +5.2 (*c* = 0.33, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.42–7.28 (m, 5 H), 6.15 (br. d, *J* = 8.3 Hz, 1 H), 5.39 (br. s, 1 H), 5.17–5.03 (m, 2 H), 4.27–4.06 (m, 2 H), 3.15 (m, 1 H), 2.15 (m, 1 H), 1.65–1.44 (m, 3 H), 1.43–1.24 (m, 4 H), 1.02–0.84 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.1, 155.8, 136.1, 128.4, 128.0, 127.8, 78.7, 66.8, 62.5, 50.6, 48.7, 41.9, 31.1, 24.6, 22.9, 22.3, 19.0, 18.7, 18.2 ppm. IR (neat): $\tilde{\nu}$ = 3392, 2956, 2926, 1740, 1648, 1517, 1462, 1367, 1249, 1063 cm^{-1}. HRMS (ESI): calcd. for $C_{20}H_{33}O_4N_2$ $[M + H]^+$ 365.2440; found 365.2444.

Benzyl [(2*S*)-1-[(4*S*)-3-Hydroxy-2,6-dimethylheptan-4-ylamino]-3-methyl-1-oxobutan-2-yl]carbamate (17c): Compound **17c** (106 mg, 53%) was prepared as a colourless oil from the coupling of **12c** (Cbz-L-Val-OH; 189.4 mg, 0.7546 mmol) with the free amine derived from **6** and **6a** (80 mg, 0.5031 mmol) following the procedure described for the synthesis of **16a**. R_f = 0.5 (30% EtOAc/hexane). $[\alpha]_D^{26}$ = +11.8 (*c* = 1.2, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.39–7.28 (m, 5 H), 6.11 (br. d, *J* = 8.6 Hz, 1 H), 5.34 (br. d, *J* = 5.7 Hz, 1 H), 5.11 (ABq, *J* = 17.2, 12.4 Hz, 2 H), 4.14 (m, 1 H), 3.94 (dd, *J* = 8.6, 6.7 Hz, 1 H), 3.15 (m, 1 H), 2.31 (br. s, 1 H), 2.13 (m, 1 H), 1.74–1.53 (m, 2 H), 1.48 (m, 1 H), 1.32 (m, 1 H), 1.03–0.85 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.9, 156.4, 136.1, 128.4, 128.1, 127.9, 78.8, 66.9, 60.7, 48.7, 42.0, 31.1, 30.8, 24.7, 22.8, 22.4, 19.3, 19.0, 18.9, 17.7 ppm. IR (neat): $\tilde{\nu}$ = 3330, 2958, 2924, 2864, 1705, 1649, 1517, 1462, 1391, 1237, 1028 cm^{-1}. HRMS (ESI): calcd. for $C_{22}H_{37}O_4N_2$ $[M + H]^+$ 393.2753; found 393.2762.

Benzyl [(2*S*,3*S*)-1-[(4*S*)-3-Hydroxy-2,6-dimethylheptan-4-ylamino]-3-methyl-1-oxopentan-2-yl]carbamate (17d): Compound **17d** (147 mg, 72%) was prepared as a white solid from the coupling of **12d** (Cbz-L-Ileu-OH; 200 mg, 0.7547 mmol) with the free amine derived from **6** and **6a** (80 mg, 0.5031 mmol) following the procedure described for the synthesis of **16a**. R_f = 0.4 (30% EtOAc/hexane). $[\alpha]_D^{26}$ = –17.4 (*c* = 0.66, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.29 (m, 5 H), 6.10 (br. d, *J* = 9.0 Hz, 1 H), 5.33 (br. d, *J* = 9.0 Hz, 1 H), 5.16–5.04 (ABq, *J* = 17.3, 12.0 Hz, 2 H), 4.13 (m, 1 H), 3.97 (m, 1 H), 3.14 (m, 1 H), 1.86 (m, 1 H), 1.70–1.64 (m, 2 H), 1.63–1.39 (m, 2 H), 1.31 (m, 1 H), 1.11 (m, 1 H), 1.00–0.83 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.0, 156.4, 136.2, 136.1, 128.4, 128.0, 127.9, 78.7, 67.0, 66.9, 60.0, 48.7, 42.0, 37.0, 31.1, 24.7, 24.5, 22.8, 22.4, 19.0, 19.0, 15.5, 11.1 ppm. IR (neat): $\tilde{\nu}$ = 3330, 2959, 2926, 2872, 1705, 1676, 1649, 1517, 1462, 1393, 1239, 1033 cm^{-1}. HRMS (ESI): calcd. for $C_{23}H_{39}O_4N_2$ $[M + H]^+$ 407.2909; found 407.2914.

Benzyl [(2*S*)-1-[(4*S*)-3-Hydroxy-2,6-dimethylheptan-4-ylamino]-1-oxo-3-phenylpropan-2-yl]carbamate (17e): Compound **17e** (200 mg, 72%) was prepared as a white solid from the coupling of **12e** (Cbz-L-Phe-OH; 282 mg, 0.9433 mmol) with the free amine derived from **6** and **6a** (100 mg, 06289 mmol) following the procedure described for the synthesis of **16a**. R_f = 0.4 (30% EtOAc/hexane), m.p. 103–105 °C. $[\alpha]_D^{26}$ = –13 (*c* = 0.46, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.41–7.15 (m, 10 H), 5.97 (br. d, *J* = 9.2 Hz, 1 H), 5.36 (br. d, *J* = 6.7 Hz, 1 H), 5.08 (ABq, *J* = 16.9, 12.2 Hz, 2 H), 4.39 (m, 1 H), 4.06 (m, 1 H), 3.17–2.95 (m, 3 H), 1.87 (m, 1 H), 1.56–1.33 (m, 2 H), 1.31–1.09 (m, 2 H), 1.02–0.78 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.4, 155.8, 136.4, 136.1, 129.2, 128.6, 128.5, 128.1, 127.9, 126.9, 78.7, 67.0, 56.6, 56.5, 48.7, 42.0, 38.4, 30.6, 24.6, 22.8, 22.3, 19.1, 18.7 ppm. IR (neat): $\tilde{\nu}$ = 3303, 2955, 2924, 2862, 1705, 1650, 1531, 1461, 1393, 1251, 1147,

1033 cm^{-1}. HRMS (ESI): calcd. for $C_{26}H_{37}O_4N_2$ $[M + H]^+$ 441.2753; found 441.2756.

Benzyl [(2*S*)-1-[(4*S*)-3-Hydroxy-2,6-dimethylheptan-4-ylamino]-4-methyl-1-oxopentan-2-yl]carbamate (17f): Compound **17f** (153 mg, 75%) was prepared as a solid from the coupling of **12** (Cbz-L-Leu-OH; 197.7 mg, 0.7547 mmol) with the free amine derived from **6** and **6a** (80 mg, 0.5031 mmol) following the procedure described for the synthesis of **16a**, m.p. 97–99 °C; R_f = 0.4 (30% EtOAc/hexane). $[\alpha]_D^{26}$ = –26.4 (*c* = 0.51, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.43–7.29 (m, 5 H), 6.33 (br. d, *J* = 7.5 Hz, 1 H), 5.28 (br. d, *J* = 8.3 Hz, 1 H), 5.16–5.03 (m, 2 H), 4.24–4.03 (m, 2 H), 3.15 (br. d, *J* = 6.0 Hz, 1 H), 1.74–1.41 (m, 6 H), 1.30 (m, 1 H), 1.06–0.82 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.9, 156.2, 136.1, 128.4, 128.1, 127.9, 78.8, 66.9, 53.7, 48.7, 41.9, 41.4, 31.1, 24.6, 22.9, 22.7, 22.4, 22.1, 22.1, 19.0, 18.8 ppm. IR (neat): $\tilde{\nu}$ = 3331, 2957, 2926, 2870, 1705, 1694, 1518, 1463, 1394, 1258, 1119, 1046 cm^{-1}. HRMS (ESI): calcd. for $C_{23}H_{39}O_4N_2$ $[M + H]^+$ 407.2909; found 407.2914.

(*S*)-Benzyl [2-(2,6-Dimethyl-4-oxoheptan-3-ylamino)-2-oxoethyl]-carbamate (18a): DMP (164 mg, 0.387 mmol) was added to a stirred solution of alcohol **16a** (113 mg, 0.3228 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The resulting solution was stirred at room temp., and the reaction was monitored by TLC. Then the reaction mixture was quenched with a mixture of saturated aq. $NaHCO_3$ and saturated aq. $Na_2S_2O_3$ (1:1), and the mixture was stirred for a further 15 min to give a clear solution. The aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography to give ketone **18a** (95 mg, 84%) as a colourless liquid. R_f = 0.4 (50% EtOAc/hexane). $[\alpha]_D^{26}$ = +44.3 (*c* = 0.49, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.41–7.28 (m, 5 H), 6.57 (br. d, *J* = 5.5 Hz, 1 H), 5.45 (br. s, 1 H), 5.14 (s, 2 H), 4.61 (dd, *J* = 8.8, 4.4 Hz, 1 H), 3.98–3.85 (m, 2 H), 2.43–2.31 (d, 2 H), 2.24–2.12 (m, 2 H), 1.01–0.72 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 208.5, 169.1, 156.5, 136.1, 128.3, 127.9, 127.8, 66.9, 62.4, 49.6, 44.3, 29.9, 24.0, 22.4, 22.3, 19.7, 16.5 ppm. IR (neat): $\tilde{\nu}$ = 3392, 1741, 1693, 1647, 1516, 1463, 1426, 1396 cm^{-1}. HRMS (ESI): calcd. for $C_{19}H_{29}O_4N_2$ $[M + H]^+$ 349.2127; found 349.2131.

Benzyl [(*S*)-1-[(*S*)-2,6-Dimethyl-4-oxoheptan-3-ylamino]-1-oxopropan-2-yl]carbamate (18b): Ketone **18b** (102 mg, 82%) was prepared as a white solid from **16b** (125 mg, 0.3434 mmol) following the procedure described for the synthesis of **18a**. R_f = 0.4 (40% EtOAc/hexane), m.p. 88–90 °C. $[\alpha]_D^{26}$ = –2.5 (*c* = 0.2, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.41–7.28 (m, 5 H), 6.63 (br. d, *J* = 7.5 Hz, 1 H), 5.43 (br. d, *J* = 6.7 Hz, 1 H), 5.11 (s, 2 H), 4.57 (q, *J* = 4.5 Hz, 1 H), 4.31 (m, 1 H), 2.42–2.30 (m, 2 H), 2.27–2.09 (m, 3 H), 1.43–1.32 (m, 3 H), 1.02–0.67 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 208.4, 172.4, 155.8, 136.1, 128.4, 128.1, 127.9, 66.9, 62.5, 50.5, 49.7, 30.1, 30.0, 24.1, 22.5, 22.4, 19.8, 18.5 ppm. IR (neat): $\tilde{\nu}$ = 3392, 1741, 1693, 1647, 1516, 1463, 1315 cm^{-1}. HRMS (ESI): calcd. for $C_{20}H_{31}O_4N_2$ $[M + H]^+$ 363.2283; found 363.2288.

Benzyl [(*S*)-1-[(*S*)-2,6-Dimethyl-4-oxoheptan-3-ylamino]-3-methyl-1-oxobutan-2-yl]carbamate (18c): Ketone **18c** (126 mg, 89%) was prepared as a white solid from **16c** (126 mg, 0.3230 mmol) following the procedure described for the synthesis of **18a**. R_f = 0.4 (20% EtOAc/hexanes), m.p. 114–116 °C. $[\alpha]_D^{26}$ = +12.4 (*c* = 0.18, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.28 (m, 5 H), 6.45 (br. d, *J* = 8.1 Hz, 1 H), 5.39 (br. d, *J* = 7.5 Hz, 1 H), 5.11 (s, 2 H), 4.59 (dd, *J* = 8.6, 4.1 Hz, 1 H), 4.05 (m, 1 H), 2.45–2.30 (m, 2 H), 2.26–2.04 (m, 3 H), 1.03–0.69 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 208.5, 171.4, 156.3, 136.2, 128.4, 128.1, 127.9, 67.0, 62.5, 60.5, 49.8, 31.0, 30.1, 24.3, 22.5, 22.3, 19.9, 19.1, 17.7, 16.6 ppm. IR (neat): $\tilde{\nu}$ = 3393, 1741, 1706, 1693, 1516, 1463, 1340, 1172 cm^{-1}. HRMS (ESI): calcd. for $C_{22}H_{35}O_4N_2$ $[M + H]^+$ 391.2596; found 391.2602.

Benzyl [(2*S*,3*S*)-1-[(*S*)-2,6-Dimethyl-4-oxoheptan-3-ylamino]-3-methyl-1-oxopentan-2-yl]carbamate (18d): Ketone **18d** (134 mg, 85%) was prepared as a white solid from **16d** (158 mg, 0.3899 mmol) following the procedure described for the synthesis of **18a**. R_f = 0.5 (20% EtOAc/hexane), m.p. 106–108 °C. $[\alpha]_D^{26}$ = +25.8 (*c* = 0.32, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.37–7.28 (m, 5 H), 6.38 (br. d, *J* = 8.7 Hz, 1 H), 5.35 (br. d, *J* = 7.6 Hz, 1 H), 5.15–5.07 (m, 2 H), 4.58 (dd, *J* = 8.7, 4.3 Hz, 1 H), 4.07 (m,1 H), 2.45–2.29 (m, 2 H), 2.23–2.13 (m, 2 H), 1.85 (m, 1 H), 1.50 (m, 1 H), 1.15 (m, 1 H), 1.00–0.87 (m, 16 H), 0.81–0.73 (m, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 208.4, 171.3, 156.1, 136.2, 128.4, 128.1, 128.0, 66.9, 62.5, 59.7, 49.8, 37.4, 30.1, 24.7, 24.3, 22.5, 22.3, 19.9, 16.7, 15.4, 11.3 ppm. IR (neat): $\tilde{\nu}$ = 3301, 2962, 1741, 1693, 1647, 1516, 1463, 1395, 1367 cm^{-1}. HRMS (ESI): calcd. for $C_{23}H_{37}O_4N_2$ $[M + H]^+$ 405.2753; found 405.2759.

Benzyl [(*S*)-1-[(*S*)-2,6-Dimethyl-4-oxoheptan-3-ylamino]-1-oxo-3-phenylpropan-2-yl]carbamate (18e): Ketone **18e** (142 mg, 92%) was prepared as a white solid from **16e** (155 mg, 0.3521 mmol) following the procedure described for the synthesis of **18a**. R_f = 0.5 (20% EtOAc/hexane), m.p. 94–96 °C. $[\alpha]_D^{25.7}$ = +6.0 (*c* = 0.33, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.46–7.12 (m, 10 H), 6.41 (br. d, *J* = 7.3 Hz, 1 H), 5.38 (br. d, 7.5, 1 H), 5.16–5.03 (m, 2 H), 4.56–4.41 (m, 2 H), 3.07 (d, *J* = 6.7 Hz, 2 H), 2.39–2.26 (m, 2 H), 2.21–2.03 (m, 2 H), 1.04–0.64 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 207.9, 170.8, 155.8, 136.1, 129.2, 128.6, 128.4, 128.1, 128.0, 127.0, 67.0, 62.6, 56.2, 49.7, 38.3, 30.1, 24.2, 22.5, 22.4, 19.8, 16.6 ppm. IR (neat): $\tilde{\nu}$ = 3298, 2958, 2926, 1740, 1692, 1650, 1531, 1462, 1395, 1368, 1286, 1039 cm^{-1}. HRMS (ESI): calcd. for $C_{26}H_{35}O_4N_2$ $[M + H]^+$ 439.2596; found 439.2599.

(*S*)-Benzyl [2-(2,6-Dimethyl-3-oxoheptan-4-ylamino)-2-oxoethyl]-carbamate (19a): Ketone **19a** (98 mg, 83%) was prepared as a yellow liquid from **17a** (119 mg, 0.340 mmol) following the procedure described for the synthesis of **18a**. R_f = 0.4 (50% EtOAc/hexane). $[\alpha]_D^{26}$ = +14.4 (*c* = 0.38, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.39–7.28 (m, 5 H), 6.55 (br. d, *J* = 8.3 Hz, 1 H), 5.48 (m, 1 H), 5.12 (s, 2 H), 4.83 (m, 1 H), 3.99–3.81 (m, 2 H), 2.79 (m, 1 H), 1.71–1.46 (m, 2 H), 1.35 (m, 1 H), 1.17–1.06 (m, 6 H), 1.00–0.86 (m, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 213.3, 168.8, 156.4, 136.1, 128.4, 128.0, 127.9, 67.0, 54.7, 44.2, 40.4, 37.9, 24.9, 23.2, 21.5, 18.8, 17.5 ppm. IR (neat): $\tilde{\nu}$ = 3333, 2960, 2924, 2856, 1708, 1676, 1658, 1517, 1462, 1394, 1242, 1048 cm^{-1}. HRMS (ESI): calcd. for $C_{19}H_{29}O_4N_2$ $[M + H]^+$ 349.2127; found 349.2133.

Benzyl [(*S*)-1-[(*S*)-2,6-Dimethyl-3-oxoheptan-4-ylamino]-1-oxopropan-2-yl]carbamate (19b): Ketone **19b** (141 mg, 90%) was prepared as a yellow liquid from **17b** (157 mg, 0.4313 mmol) following the procedure described for the synthesis of **18a**. R_f = 0.4 (40% EtOAc/hexane). $[\alpha]_D^{25.7}$ = –11.7 (*c* = 0.53, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.29 (m, 5 H), 6.50 (br. d, *J* = 7.5 Hz, 1 H), 5.37 (br. d, *J* = 6.0 Hz, 1 H), 5.11 (s, 2 H), 4.79 (m, 1 H), 4.27 (m, 1 H), 2.80 (m, 1 H), 1.68–1.47 (m, 2 H), 1.43–1.30 (m, 4 H), 1.19–1.04 (m, 6 H), 1.00–0.84 (m, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 213.1, 172.1, 155.7, 136.0, 128.3, 127.9, 127.7, 66.6, 54.6, 50.2, 40.1, 37.7, 24.7, 23.1, 21.5, 18.7, 17.5 ppm. IR (neat): $\tilde{\nu}$ = 3314, 2962, 2931, 1707, 1675, 1657, 1517, 1462, 1244, 1066, 1038 cm^{-1}. HRMS (ESI): calcd. for $C_{20}H_{31}O_4N_2$ $[M + H]^+$ 363.2283; found 363.2288.

Benzyl [(*S*)-1-[(*S*)-2,6-Dimethyl-3-oxoheptan-4-ylamino]-3-methyl-1-oxobutan-2-yl]carbamate (19c): Ketone **19c** (85 mg, 80%) was prepared as a white solid from **17c** (106 mg, 0.2704 mmol) following the procedure described for the synthesis of **18a**. R_f = 0.5 (20% EtOAc/hexane), m.p. 129–131 °C. $[\alpha]_D^{26}$ = +3.8 (*c* = 0.92, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.42–7.28 (m, 5 H), 6.44 (br. d, *J* = 4.7 Hz, 1 H), 5.42 (br. d, *J* = 8.4 Hz, 1 H), 5.11 (s, 2 H), 4.83 (m, 1 H), 4.05 (m, 1 H), 2.80 (m, 1 H), 2.12 (m, 1 H), 1.63 (m, 1 H), 1.51 (m, 1 H), 1.37 (m, 1 H), 1.19–1.04 (m, 6 H), 1.01–0.85 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 213.1, 170.9, 156.2, 128.4, 128.0, 127.9, 66.9, 60.1, 54.6, 40.6, 38.1, 31.3, 24.9, 23.3, 21.6, 19.0, 18.9, 17.7, 17.5 ppm. IR (neat): $\tilde{\nu}$ = 3302, 2962, 2928, 2874, 1707, 1650, 1531, 1462, 1388, 1374, 1287, 1238, 1100, 1035 cm^{-1}. HRMS (ESI): calcd. for $C_{22}H_{29}O_4N_2$ $[M + H]^+$ 391.2596; found 391.2601.

Benzyl [(2*S*,3*S*)-1-[(*S*)-2,6-Dimethyl-3-oxoheptan-4-ylamino]-3-methyl-1-oxopentan-2-yl]carbamate (19d): Ketone **19d** (129 mg, 88%) was prepared as a white solid from **17d** (147 mg, 0.362 mmol) following the procedure described for the synthesis of **18a**. R_f = 0.4 (20% EtOAc/hexane), m.p. 119–121 °C. $[\alpha]_D^{26}$ = –2.3 (*c* = 0.53, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.39–7.29 (m, 5 H), 6.29 (br. d, *J* = 7.5 Hz, 1 H), 5.35 (br. d, *J* = 8.3 Hz, 1 H), 5.10 (s, 2 H), 4.81 (m, 1 H), 4.04 (m, 1 H), 2.79 (m, 1 H), 1.83 (m, 1 H), 1.71–1.57 (m, 2 H), 1.56–1.43 (m, 2 H), 1.36 (m, 1 H), 1.17–1.06 (m, 6 H), 0.99–0.83 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 213.0, 170.9, 156.1, 136.2, 128.4, 128.0, 127.9, 66.9, 59.5, 54.6, 40.6, 38.1, 37.6, 24.9, 24.7, 23.3, 21.6, 18.8, 17.5, 15.3, 11.3 ppm. IR (neat): $\tilde{\nu}$ = 3283, 2962, 2924, 1741, 1693, 1646, 1516, 1463, 1395, 1367, 1037 cm^{-1}. HRMS (ESI): calcd. for $C_{23}H_{37}O_4N_2$ $[M + H]^+$ 405.2753; found 405.2757.

Benzyl [(*S*)-1-[(*S*)-2,6-Dimethyl-3-oxoheptan-4-ylamino]-1-oxo-3-phenylpropan-2-yl]carbamate (19e): Ketone **19e** (188 mg, 94%) was prepared as a white solid from **17e** (200 mg, 0.4545 mmol) following the procedure described for the synthesis of **18a**. R_f = 0.4 (20% EtOAc/hexanes), m.p. 119–121 °C. $[\alpha]_D^{26}$ = –5.7 (*c* = 0.35, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.42–7.12 (m, 10 H), 6.25 (br. d, *J* = 8.3 Hz, 1 H), 5.31 (br. d, *J* = 7.5 Hz, 1 H), 5.15–5.05 (m, 2 H), 4.74 (m, 1 H), 4.44 (m, 1 H), 3.17–2.98 (m, 2 H), 2.72 (m, 1 H), 1.57–1.18 (m, 3 H), 1.15–1.03 (m, 6 H), 0.97–0.82 (m, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 212.4, 170.3, 155.7, 136.1, 129.2, 128.6, 128.5, 128.1, 127.9, 127.0, 67.0, 56.0, 54.6, 40.8, 38.4, 37.9, 24.8, 23.2, 21.7, 18.9, 17.5 ppm. IR (neat): $\tilde{\nu}$ = 3280, 2961, 2924, 1741, 1647, 1517, 1463, 1426, 1395, 1340, 1263, 1051 cm^{-1}. HRMS (ESI): calcd. for $C_{26}H_{35}O_4N_2$ $[M + H]^+$ 439.2596; found 439.2599.

Benzyl [(*S*)-1-[(*S*)-2,6-Dimethyl-3-oxoheptan-4-ylamino]-4-methyl-1-oxopentan-2-yl]carbamate (19f): Ketone **19f** (131 mg, 86%) was prepared as a white solid from **17f** (153 mg, 0.3768 mmol) following the procedure described for the synthesis of **18a**. R_f = 0.4 (20% EtOAc/hexanes), m.p. 125–127 °C. $[\alpha]_D^{26}$ = –7.9 (*c* = 0.6, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.29 (m, 5 H), 6.39 (br. d, *J* = 8.3 Hz, 1 H), 5.22–5.08 (m, 3 H), 4.79 (m, 1 H), 4.20 (m, 1 H), 2.80 (m, 1 H), 1.72–1.42 (m, 6 H), 1.18–1.06 (m, 6 H), 1.00–0.86 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 213.0, 171.9, 156.0, 136.1, 128.4, 128.0, 127.9, 66.9, 54.7, 53.4, 41.5, 40.5, 37.9, 24.9, 24.5, 23.3, 22.8, 21.9, 21.6, 18.9, 17.5 ppm. IR (neat): $\tilde{\nu}$ = 3297, 2959, 2928, 2868, 1694, 1647, 1545, 1461, 1266, 1248, 1126, 1048 cm^{-1}. HRMS (ESI): calcd. for $C_{23}H_{37}O_4N_2$ $[M + H]^+$ 405.2753; found 405.2756.

(5*R*,6*S*)-5-Isobutyl-6-isopropylpiperazin-2-one (20a): A solution of ketone **18a** (95 mg, 0.2743 mmol) in MeOH (3 mL) was hydrogenated at balloon pressure (1 atm) in the presence of 10% Pd/C (95 mg). The reaction was monitored by TLC. The mixture was filtered through a pad of Celite, which was then washed with EtOAc. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography to give cyclic compound **20a** (42 mg, 77%) as a pale yellow liquid. R_f = 0.2 (EtOAc). $[\alpha]_D^{26}$ = –9.4 (*c* = 0.45, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.09 (br. s, 1 H), 3.53–3.44 (m, 2 H), 3.22–3.08 (m, 2 H), 1.80–1.66 (m, 2 H), 1.52 (m, 1 H), 1.09 (m, 1 H), 1.02–0.85 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.9, 57.2, 51.4, 47.7, 32.6, 28.7, 23.9, 23.6, 21.1, 19.3, 18.4 ppm. IR (neat): $\tilde{\nu}$ = 3362, 2960, 2928, 1646, 1367, 1016 cm^{-1}. HRMS (ESI): calcd. for $C_{11}H_{23}ON_2$ $[M + H]^+$ 199.1810; found 199.1812.

(3*S*,5*R*,6*S*)-5-Isobutyl-6-isopropyl-3-methylpiperazin-2-one (20b): Cyclic compound **20b** (43 mg, 72%) was prepared as a pale yellow solid from **18b** (102 g, 0.2817 mmol) following the procedure described for the synthesis of **20a**. R_f = 0.4 (70% EtOAc/hexane), m.p. 80–82 °C. $[\alpha]_D^{26}$ = –29.1 (*c* = 0.3, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.16 (br. s, 1 H), 3.52 (q, *J* = 6.7 Hz, 1 H), 3.20 (m, 1 H), 3.09 (m, 1 H), 1.93 (m, 1 H), 1.66 (m, 1 H), 1.41–1.21 (m, 5 H), 1.04–0.85 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.0, 59.6, 54.5, 53.3, 40.6, 27.8, 24.7, 23.1, 22.4, 21.5, 18.2, 17.6 ppm. IR (neat): $\tilde{\nu}$ = 3278, 2959, 2929, 1648, 1369 cm^{-1}. HRMS (ESI): calcd. for $C_{12}H_{23}ON_2$ $[M – H]^+$ 211.1810; found 211.1812.

(3*S*,5*R*,6*S*)-5-Isobutyl-3,6-diisopropylpiperazin-2-one (20c): Cyclic compound **20c** (50 mg, 64%) was prepared as a liquid from **18c** (126 mg, 0.3230 mmol) following the procedure described for the synthesis of **20a**. R_f = 0.4 (30% EtOAc/hexane). $[\alpha]_D^{26}$ = –44.7 (*c* = 0.24, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.61 (br. s, 1 H), 3.25 (d, *J* = 2.7 Hz, 1 H), 2.67 (dt, *J* = 9.1, 2.7 Hz, 1 H), 2.48 (m, 1 H), 1.93 (m, 1 H), 1.79 (m, 1 H), 1.37–1.22 (m, 3 H), 1.07–0.81 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.3, 63.6, 63.3, 52.7, 40.9, 29.1, 27.6, 24.5, 23.8, 21.3, 19.8, 19.3, 16.5, 14.3 ppm. IR (neat): $\tilde{\nu}$ = 3209, 2959, 2925, 2863, 1659, 1463, 1339 cm^{-1}. HRMS (ESI): calcd. for $C_{14}H_{29}ON_2$ $[M + H]^+$ 241.2279; found 241.2278.

(3*S*,5*R*,6*S*)-3-*sec*-butyl-5-isobutyl-6-isopropylpiperazin-2-one (20d): Cyclic compound **20d** (48 mg, 56%) was prepared as a liquid from **18d** (134 mg, 0.3353 mmol) following the procedure described for the synthesis of **20a**. R_f = 0.35 (30% EtOAc/hexane). $[\alpha]_D^{26}$ = –31.8 (*c* = 0.4, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.58 (br. s, 1 H), 3.28 (d, *J* = 2.0 Hz, 1 H), 3.00 (dd, *J* = 8.9, 2.0 Hz, 1 H), 2.65 (dt, *J* = 9.9, 2.9 Hz, 1 H), 2.18 (m, 1 H), 1.93 (m, 1 H), 1.80 (m, 1 H), 1.44 (m, 1 H), 1.36–1.21 (m, 3 H), 1.04–0.82 (m, 18 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.2, 63.9, 63.4, 52.9, 40.9, 36.1, 27.6, 24.5, 24.4, 23.9, 21.3, 19.8, 16.1, 14.3, 12.3 ppm. IR (neat): $\tilde{\nu}$ = 3234, 2962, 2874, 1648, 1368 cm^{-1}. HRMS (ESI): calcd. for $C_{15}H_{29}ON_2$ $[M – H]^+$ 253.2279; found 253.2282.

(3*S*,5*R*,6*S*)-3-Benzyl-5-isobutyl-6-isopropylpiperazin-2-one (20e): Cyclic compound **20e** (75 mg, 80%) was prepared as a liquid from **18e** (142 mg, 0.3239 mmol) following the procedure described for the synthesis of **20a**. R_f = 0.4 (50% EtOAc/hexane). $[\alpha]_D^{26}$ = –59.4 (*c* = 0.29, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35–7.17 (m, 5 H), 6.41 (br. s, 1 H), 3.64 (dd, *J* = 8.3, 3.7 Hz, 1 H), 3.33 (dd, *J* = 13.5, 3.0 Hz, 2 H), 3.16–3.01 (m, 2 H), 2.93 (dd, *J* = 13.5, 8.3 Hz, 1 H), 1.83 (m, 1 H), 1.50 (m, 1 H), 1.35–1.14 (m, 2 H), 0.98–0.69 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.7, 138.1, 129.5, 128.3, 126.4, 59.9, 59.0, 53.3, 40.6, 37.8, 27.6, 24.6, 22.6, 22.6, 21.5, 17.1 ppm. IR (neat): $\tilde{\nu}$ = 3217, 2957, 2927, 2870, 1659, 1562, 1516, 1476, 1426, 1276 cm^{-1}. HRMS (ESI): calcd. for $C_{18}H_{29}ON_2$ $[M + H]^+$ 289.2274; found 289.2274.

(5*R*,6*S*)-6-Isobutyl-5-isopropylpiperazin-2-one (21a): Cyclic compound **21a** (45 mg, 80%) was prepared as a colourless liquid from **19a** (98 mg, 0.2822 mmol) following the procedure described for

the synthesis of **20a**. R_f = 0.2 (EtOAc). $[\alpha]_D^{26}$ = –45.8 (*c* = 0.12, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.13 (br. s, 1 H), 3.54 (br. d, *J* = 6.0 Hz, 2 H), 3.43 (m, 1 H), 2.55 (dd, *J* = 9.8, 3.3 Hz, 1 H), 1.72 (m, 1 H), 1.63–1.50 (m, 2 H), 1.13 (m, 1 H), 1.04 (d, *J* = 6.4 Hz, 3 H), 0.98 (d, *J* = 6.4 Hz, 3 H), 0.91 (d, *J* = 6.6 Hz, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.5, 62.4, 51.1, 49.9, 38.0, 28.6, 24.2, 23.7, 20.8, 20.3, 19.2 ppm. IR (neat): $\tilde{\nu}$ = 3220, 2958, 2928, 2874, 1692, 1248 cm^{-1}. HRMS (ESI): calcd. for $C_{11}H_{23}ON_2$ $[M + H]^+$ 199.1810; found 199.1811.

(3*S*,5*R*,6*S*)-6-Isobutyl-5-isopropyl-3-methylpiperazin-2-one (21b): Cyclic compound **21b** (65 mg, 78%) was prepared as a colourless liquid from **19b** (141 mg, 0.3895 mmol) following the procedure described for the synthesis of **20a**. R_f = 0.4 (70% EtOAc/hexane). $[\alpha]_D^{26}$ = –86.3 (*c* = 0.11, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.39 (br. s, 1 H), 3.52 (q, *J* = 6.7 Hz, 1 H), 3.38 (m, 1 H), 2.62 (dd, *J* = 9.8, 3.0 Hz, 1 H), 1.79–1.50 (m, 3 H), 1.36 (d, *J* = 6.7 Hz, 3 H), 1.13 (m, 1 H), 1.03 (d, *J* = 6.0 Hz, 3 H), 0.96 (d, *J* = 6.7 Hz, 3 H), 0.89 (d, *J* = 6.0 Hz, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.5, 62.5, 55.0, 51.5, 38.5, 28.4, 24.2, 23.8, 20.8, 20.2, 19.0, 18.4 ppm. IR (neat): $\tilde{\nu}$ = 3277, 2964, 2931, 2875, 1650, 1236 cm^{-1}. HRMS (ESI): calcd. for $C_{12}H_{25}N_2O$ $[M + H]^+$ 213.1967; found 213.1972.

(3*S*,5*R*,6*S*)-6-Isobutyl-3,5-diisopropylpiperazin-2-one (21c): Cyclic compound **21c** (45 mg, 86%) was prepared as a colourless liquid from **19c** (85 mg, 0.2179 mmol) following the procedure described for the synthesis of **20a**. R_f = 0.35 (40% EtOAc/hexane). $[\alpha]_D^{26}$ = –64.7 (*c* = 0.49, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.37 (br. s, 1 H), 3.38 (d, *J* = 2.2 Hz, 1 H), 2.56 (dd, *J* = 9.9, 3.3 Hz, 1 H), 2.44 (m, 1 H), 1.76–1.65 (m, 3 H), 1.59 (m, 1 H), 1.11 (m, 1 H), 1.05–0.85 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.9, 64.0, 62.1, 51.1, 38.4, 29.8, 28.4, 24.3, 23.7, 20.9, 20.0, 19.0, 18.9, 16.4 ppm. IR (neat): $\tilde{\nu}$ = 3198, 2956, 1661, 1159, 1079 cm^{-1}. HRMS (ESI): calcd. for $C_{14}H_{29}ON_2$ $[M + H]^+$ 241.2274; found 241.2272.

(3*S*,5*R*,6*S*)-3-*sec*-Butyl-6-isobutyl-5-isopropylpiperazin-2-one (21d): Cyclic compound **21d** (50 mg, 62%) was prepared as a liquid from **19d** (129 mg, 0.319 mmol) following the procedure described for the synthesis of **20a**. R_f = 0.4 (30% EtOAc/hexane). $[\alpha]_D^{26}$ = –45.0 (*c* = 0.4, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.37 (br. s, 1 H), 3.39 (s, 1 H), 3.34 (m, 1 H), 2.54 (dd, *J* = 10.4, 2.7 Hz, 1 H), 2.12 (br. s, 1 H), 1.74–1.63 (m, 2 H), 1.57 (m, 1 H), 1.41 (m, 1 H), 1.32 (m, 1 H), 1.10 (m, 1 H), 1.03–0.98 (m, 6 H), 0.97–0.93 (m, 3 H), 0.92–0.86 (m, 9 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.3, 64.3, 62.3, 51.0, 38.4, 36.8, 28.3, 24.2, 23.7, 20.9, 20.0, 18.9, 15.7, 12.3 ppm. IR (neat): $\tilde{\nu}$ = 3206, 2958, 2852, 1657, 1395, 1367 cm^{-1}. HRMS (ESI): calcd. for $C_{15}H_{31}N_2O$ $[M + H]^+$ 255.2430; found 255.2428.

(3*S*,5*R*,6*S*)-3-Benzyl-6-isobutyl-5-isopropylpiperazin-2-one (21e): Cyclic compound **21e** (94 mg, 76%) was prepared as a colourless liquid from **19e** (188 mg, 0.4292 mmol) following the procedure described for the synthesis of **20a**. R_f = 0.35 (50% EtOAc/hexane). $[\alpha]_D^{26}$ = –33.3 (*c* = 0.15, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35–7.20 (m, 5 H), 6.54 (br. s, 1 H), 3.69 (t, *J* = 6.0 Hz, 1 H), 3.29 (m, 1 H), 3.15 (d, *J* = 6.0 Hz, 2 H), 2.53 (dd, *J* = 9.8, 3.0 Hz, 1 H), 1.59–1.33 (m, 2 H), 0.99–0.87 (m, 4 H), 0.85–0.75 (m, 10 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.5, 137.7, 129.6, 128.3, 126.5, 61.9, 59.7, 51.2, 38.0, 37.4, 28.5, 24.0, 23.6, 20.7, 20.1, 19.0 ppm. IR (neat): $\tilde{\nu}$ = 3305, 1741, 1693, 1647, 1516, 1463, 1426, 1395, 1367, 1315 cm^{-1}. HRMS (ESI): calcd. for $C_{18}H_{29}ON_2$ $[M + H]^+$ 289.2279; found 289.2281.

(3*S*,5*R*,6*S*)-3,6-Diisobutyl-5-isopropylpiperazin-2-one (21f): Cyclic compound **21f** (68 mg, 82%) was prepared as a colourless liquid from **19f** (131 g, 0.3242 mmol) following the procedure described for the synthesis of **20a**. R_f = 0.35 (30% EtOAc/hexane). $[\alpha]_D^{26}$ = –22.5 (*c* = 0.2, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.86 (br. s, 1 H), 3.39–3.25 (m, 2 H), 2.40 (dd, *J* = 9.6, 2.4 Hz, 1 H), 1.92 (m, 1 H), 1.85–1.60 (m, 2 H), 1.47 (m, 1 H), 1.35–1.21 (m, 3 H), 1.08–0.82 (m, 18 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.5, 62.6, 57.2, 53.7, 42.1, 40.3, 27.3, 24.6, 23.9, 23.9, 23.5, 21.0, 21.0, 20.6, 15.0 ppm. IR (neat): $\tilde{\nu}$ = 3203, 2956, 2926, 2869, 1660, 1309, 1159 cm^{-1}. HRMS (ESI): calcd. for $C_{15}H_{31}ON_2$ $[M + H]^+$ 255.2436; found 255.2434.

Biological Evaluation

Materials and Methods: Cancer cell lines (A549, SK-OV-3, DU-145, MDA-MB-231, MCF-7) and non-cancer cell lines (HEK-293 and NIH/3T3) used in the evaluation were acquired from ATCC (American Type Cell Culture). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye and Annexin V were purchased from Sigma–Aldrich. An ELISA (enzyme-linked immunosorbent assay) plate reader was used to record the absorbance in the MTT assay.

Cell Culture: Cells were kept in RPMI (Roswell Park Memorial Institute medium) 1640 constituting Fetal Bovine Serum (10%) and antibiotic and antimycotic solution (1%) under optimal growth conditions (temperature: 37 °C, humidity: 95%, and CO_2: 5%). The stock solution was prepared in molecular grade DMSO. The desired concentrations (2.5, 5, 10, 20, 50, and 100 μM) of the compounds were obtained by diluting the stock solution with culture media before addition to the cells.

In-vitro Cytotoxicity Assay: The compound corresponding to the proposed structure of piperazirum was tested, along with its stereoisomers and analogues, for in vitro anticancer activity across different cancer cell lines (A549, SK-OV-3, DU145, MDA-MB-231, MCF-7) and non-cancer cell lines (HEK-293 and NIH/3T3) using a colorimetric MTT assay. Briefly, cells were incubated in 96-well plates (4×10^3 cells/well) for 24 h. After 24 h, cells were treated with the test compound at concentrations of 2.5, 5, 10, 20, 50, and 100 μM, and the resulting mixtures were incubated for 48 h. At the end of the incubation period, MTT (10 mg/mL; 10 μL) was added to each well, and the mixtures were incubated for a further 3 h. The intensity of purple coloured water-insoluble formazan dye [solubilized by the addition of DMSO (100 μL) to each well] formed due to the reduction of MTT by living cells was recorded using an ELISA plate reader at 540 nm. All the experiments were performed at least three times independently, and IC_{50} values were calculated using Graph pad prism software.

Supporting Information (see footnote on the first page of this article): Copies of the ^{1}H and ^{13}C NMR spectra of all new compounds, and crystallographic data for compound **15**.

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