The Synthesis of Two Furan-Based Analogues of the α′,β′-Epoxy Ketone Proteasome Inhibitor Eponemycin

Bibia Bennacer,[a] Dominique Trubuil,[a] Christian Rivalle,*[a] and David S. Grierson[a]

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Myers's methodology for enantioselective amino acid synthesis was employed to prepare the *N*-Boc didehydroleucine amide derivative **15** and to effect its conversion into the acylfuran intermediate **17**. Coupling of **19** (R = H) with *N*-(isooctanoyl)serine provided the furan-based analogue **4** of eponemycin (*de* = 96 %), a peptide epoxide with potent cytotoxic and anti-angiogenesis properties. In an identical fashion the corresponding unsaturated analogue **5** of eponemycin was prepared (*de* = 48 %).

Introduction

Eponemycin **1** is a potent peptide based cytotoxic agent, isolated in 1989 by Sugawara et al.[1] from *Streptomyces hygroscopicus* (IC_{50} = 4 nM in B16 and 25 nM in L1210). Distinguishing features in this molecule are an unnatural γ,δ-dehydroleucine residue, an N-terminal lipophilic isooctanoic acid component, and a reactive C-terminal α′,β′-epoxy ketone motif. Eponemycin is also a potent angiogenesis inhibitor, resulting apparently from a cooperative inhibition of endothelial cell proliferation (IC_{50} = 77 nM) and migration (750 nM). In the in vivo assay involving the chorioallantoic membrane of a growing chick embryo a dose-dependent inhibition was observed with a ID_{50} of 0.1 ng, or 0.25 picomol/egg.[2] As this dose is higher than that required for antitumor activity, eponemycin represents a potentially interesting lead in the search for agents which block tumor angiogenesis.

In 1999 Crews et al.[3] showed by affinity chromatography that eponemycin binds through covalent bond formation to crucial N-terminal Thr residues in the catalytic site of two protease β-sub-units (LMP2 and LMP7) of proteasome 20S (the proteolytic core of proteasome 26S), thereby inhibiting the protease activity of the complex. For epoxomicin (**2**)[4,5] (a related peptide proteasome inhibitor), it was demonstrated that the sensitive epoxy ketone motif in the molecule reacts with both the hydroxy and amino groups in the catalytic Thr residues with formation of a morpholino ring. Furthermore, it has been shown that the C-2(*S*) isomer of **2** is more than 100 times less potent as an inhibitor of proteasome activity than the C-2(*R*) isomer. It is reasonable to assume that eponemycin inhibits the proteasome in an identical fashion.

In view of the finding that the expression of many factors associated with angiogenesis (integrins $\alpha_v\beta_3$ and certain growth factors, etc.) are indirectly regulated by the proteasome,[6,7] its inhibition will have an impact on capillary formation. Indeed, it has been shown that the proteasome 20S inhibitor lactacystin is a potent anti-angiogenesis agent,[8] acting through inhibition of the expression of $\alpha_v\beta_3$ adhesion proteins on the surface of endothelial cells.

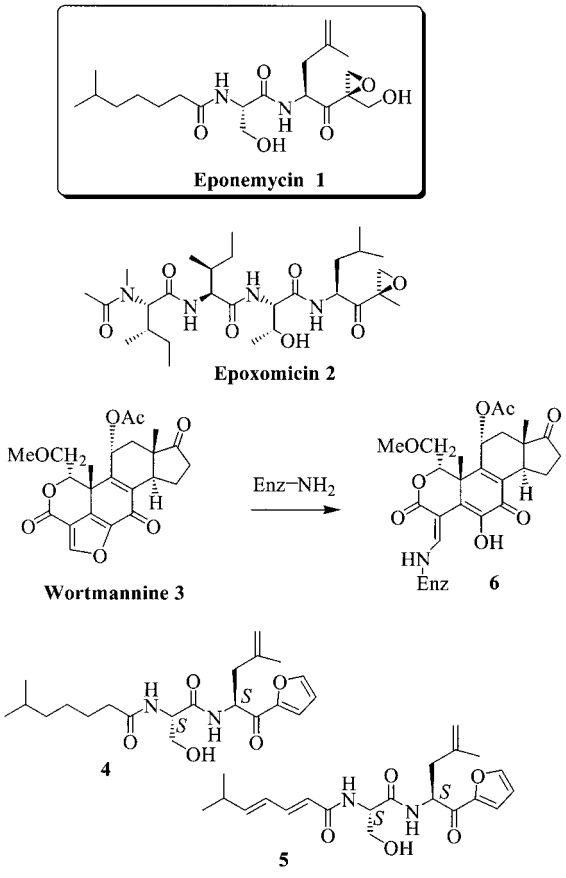
Results and Discussion

In the context of research on the development of anti-angiogenesis agents, one objective is to design eponemycin analogues which are more stable than the natural product, but maintain their capacity to react with the nucleophilic OH/NH_2 functions in the Thr residue in the targeted proteases in Proteasome 20S. It was hoped that for molecules of this type it might be possible to dissociate the desired anti-angiogenesis properties of **1** from its pronounced cytotoxicity.

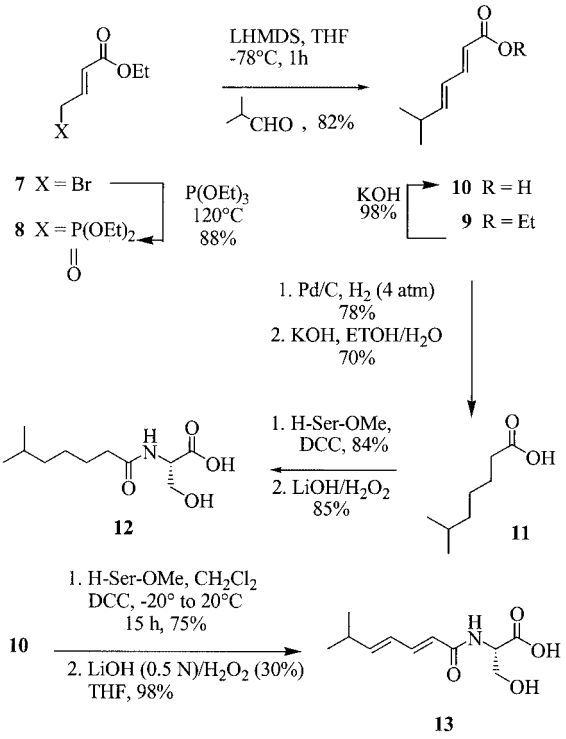
Taking our inspiration from the mechanism of action of wortmannine **3**, a PI 3 kinase inhibitor,[9,10] we have directed attention to the synthesis of the furan analogues **4** and **5** of eponemycin. In wortmannine the acylfuran reacts with a nitrogen nucleophile by an addition−elimination process, which produces a ring-opened Michael adduct (cf. **6**). This intermediate subsequently ring-closes to produce a "pyrrole analogue" of **3** which is bound to the enzyme. It is noteworthy that because compounds **4** and **5** lack the C-2 stereocenter, their specificity and affinity for the proteasome is an open question. This opening of the adduct has also been observed by reaction with alkylamines.[11]

The two isooctanoic acid components required for the synthesis of furan analogues **4** and **5** were prepared in a

[a] UMR 176 CNRS-Institut Curie, Laboratoire de Pharmacochimie
Bat. 110 Centre Universitaire, 91405 Orsay, France
Fax: (internat.) + 33-(0)1-69075381
E-mail: rivalle@curie.u-psud.fr



Scheme 1



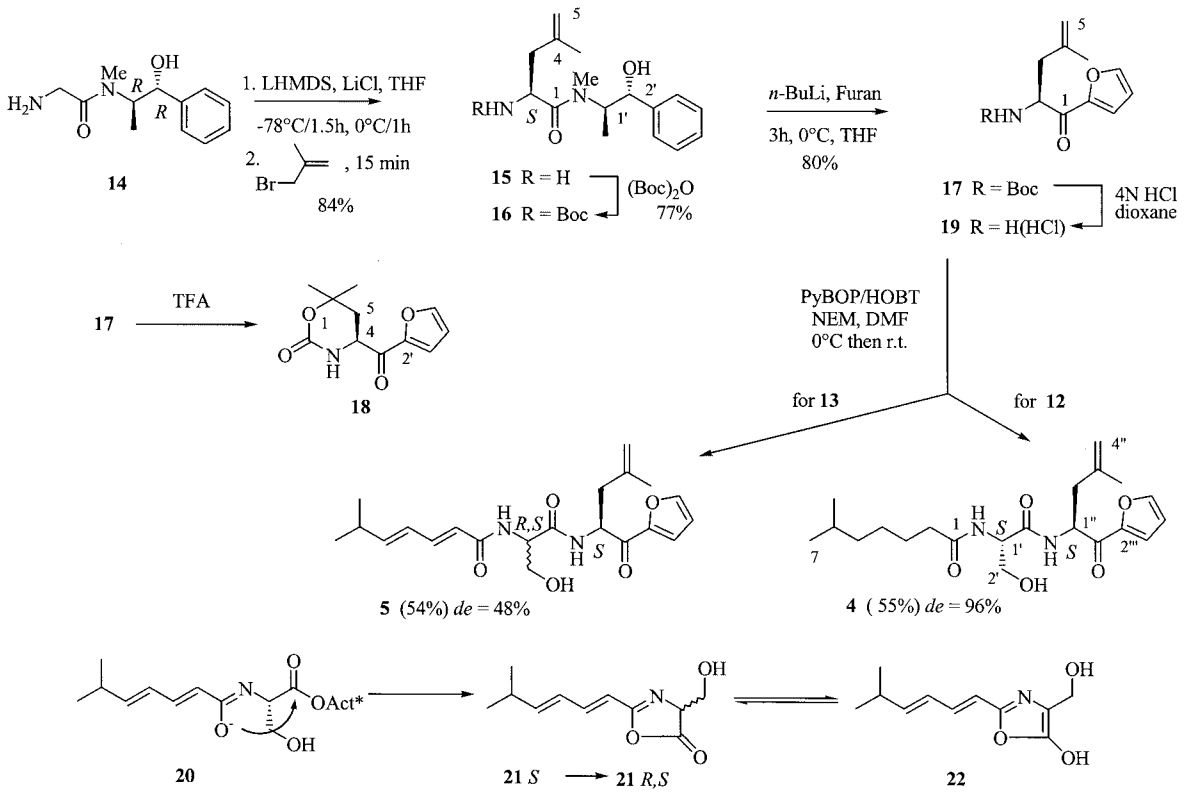
Scheme 2

straightforward fashion by reaction of the phosphonate **8** [obtained from ethyl 4-bromocrotonate (**7**)] with isobutyraldehyde under Horner–Emmons conditions.[12,13] This was followed either by (i) direct hydrolysis of the dienoate intermediate **9** (only the *trans* isomer was obtained) to the acid **10**, or (ii) double bond reduction by catalytic hydrogenation prior to ester saponification, to give **11**. Both compounds **10** and **11** were coupled with H-Ser-OMe using DCC, and the derived serine derivatives were treated with $LiOH/H_2O_2$ to give the two "left side"components **12** and **13** in high yield and high enantiomeric purity.

The principle challenge in the construction of the eponemycin analogues **4** and **5** is to make available multigram quantities of a suitable derivative of the unatural amino acid L-(2*S*)-didehydroleucine. There exists today a wide range of chiral auxillary and chiral-medium-based strategies for the enantioselective synthesis of amino acids.[14–17]] We opted to use the approach developed by Myers et al. that gives access to L-amino acids through stereoselective alkylation of (1*R*,2*R*)-(–)-pseudoephedrine glycinamide.[18–25] For our purposes, an attractive feature of this methodology is the possibility of displacing the chiral auxillary by reaction with organometallic reagents, which ought to provide direct access to a range of α-amino ketone products.

Following Myers's protocol, glycinamide **14** was treated with 1.95 equivalents of *n*BuLi (or LDA) and dry LiCl in THF at −78 °C.[21] The dianion thus generated was then treated in situ with 3-bromo-2-methylpropene at 0 °C . Under these conditions the yield of **15** never exceeded 30 %. This situation was not improved even by taking strict measures to dry the starting materials (especially **14**), or by dosing the percentage of residual water of crystallization in **14** and adding the appropriate excess of lithium base. However, by employing the more hindered base lithium bis(trimethylsilyl)amide (LHMDS), the possible problems of residual water and competing *N*-alkylation/addition of the base to the carboxamide function were alleviated.[24] Under these conditions (using only a slight excess of LHMDS) the alkylation product **15** was obtained on a 10-gram scale as a viscous pale yellow oil in 84 % yield after flash column chromatography.

Compound **15** was subsequently converted into the corresponding *N*-Boc derivative **16** by treatment with $(Boc)_2O$ (small quantities of *O*-Boc contaminants were destroyed before workup by simply stirring overnight with K_2CO_3 in MeOH). In the next (crucial) step, the reaction of this *N*-Boc intermediate with an excess (3.5 equiv.) of 2-furyllithium in THF at 0 °C led to the formation of the acylfuran **17**, isolated as white crystals in 80 % yield, and in 96–97 % *ee* (as determined by chiral column HPLC). Removal of the *N*-Boc group was attempted using TFA in CH_2Cl_2, *p*TsOH in CH_2Cl_2, and concd. HCl in EtOAc, but the oxazin-2-one **18** was obtained in each case. However, the formation of this cationic cyclization product could be minimized (to the extent of 10–20 %) by treatment of **17** with 4 N HCl in dioxane (20 °C, 2 h). Amine **19** was obtained in 80 % yield without any loss of optical purity.



Scheme 3

Finally, in the coupling reaction of amine **19** with the serine derivative **12** {[(benzotriazol-1-yl)oxy]tripyrrolidinophosphonium hexafluorophosphate/1-hydroxybenzotriazole, PyBOP/HOBT} the choice of the amine base used to free the amine from its HCl salt was critical. When Et_3N, DIEA (Hünig′s base), or NMM were employed the diastereoselectivities obtained were in the range 35−70 %. Fortunately, when *N*-ethyl morpholine (NEM) was used, the acylfuran analogue **4** of eponemycin was obtained in 55 % yield and with 96 % *de*.

In the corresponding coupling reaction of the unsaturated serine derivative **13** with amine **19**, using NEM as base, the acylfuran analogue **5** was produced in only 48 % *de*. As the only difference between the two coupling reactions was the nature of the serine component, we suspected that the C-1′ center in **5** had undergone partial epimerization. This was demonstrated to be the case by studying the analogous coupling of **13** with glycine methyl ester using chiral column HPLC. In this reaction we observed a maximum enantiomeric excess of 58 %.

A plausible rationale for this loss of optical purity could be that there is a significant concentration of the azatriene anion **20** in the reaction medium . The negatively charged oxygen thus has ample opportunity to react with activated carboxylic acid to give the cyclic intermediate **21**. Conversion of **21** to its tautomeric oxazole form **22** results in a scrambling of the serine stereocenter. As compound **21** is also an activated ester, it may be involved in the coupling with **19**. An analogous epimerisation via an oxazole was observed by Griehl et al. during their synthesis of Boc-Ala-Ser-OH using the carbodiimide method.[26]

The antiangiogenesis activity of analogues **4** and **5** was evaluated by the rat aortic ring test.[27] The IC_{50} values found (20 to 30 nM) are of the same magnitude as those for the cytotoxic effect of these molecules measured in the L1210 cell line.[28] The proteasome inhibition properties of these eponemycin analogues have not yet been determined.

Experimental Section

General Remarks: Unless otherwise stated, all reactions were carried out under argon with dry, freshly distilled solvents, flame-dried glassware, and magnetic stirring. All solvents were reagent grade. Diethyl ether and tetrahydrofuran (THF) were distilled from sodium/benzophenone under argon. Acetonitrile and dichloromethane (CH_2Cl_2) were distilled from calcium hydride. Triethylamine was distilled from calcium hydride and stored over potassium hydroxide. *N,N*-dimethylformamide (DMF) was purchased from Aldrich and used without purification unless otherwise noted. All reactions were monitored by thin layer chromatography (TLC) using E. Merk $60F_{254}$ precoated silica gel plates. Flash column chromatography was performed with the indicated solvents and using E. Merk silica gel 60 (particle size 0.035−0.070 mm unless otherwise stated). Yields refer to chromatographically and spectroscopically pure compounds, except where indicated otherwise. Melting points were taken with a Kofler melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin−Elmer 341 polarimeter at the sodium D line (589 nm) and are reported as follows: $[\alpha]_D^{20}$ (*c* in g/100 mL, solvent). Infra-red spectra were re-

corded with a Perkin−Elmer 1710FT spectrophotometer. ^{1}H NMR spectra were recorded with a Bruker AC-200 (200 MHz) or with a Bruker AC-300 (300 MHz) spectrometer at ambient temperature using an internal deuterium lock. Chemical shifts are referenced to residual chloroform (δ = 7.24 ppm) or residual methanol (δ = 4.78 ppm). ^{13}C NMR spectra were recorded with either a Bruker AC-200 (50 MHz) spectrometer or a Bruker AC-300 (75 MHz) spectrometer at ambient temperature using an internal deuterium lock. Chemical shifts are referenced to chloroform (δ = 77.0 ppm) or methanol (δ = 49.0 ppm). High and low resolution mass spectra were carried out by the I.C.M.O. Mass Spectrometry Service at the University of Paris XI. Microanalyses were performed by the I.C.S.N.-C.N.R.S. Elemental Analysis Center at Gif-sur-Yvette. High-performance liquid chromatography (HPLC) was performed with a Waters component analytical system by the I.C.S.N.-C.N.R.S. HPLC Center at Gif-sur-Yvette.

Ethyl (2*E*)-4-(Diethoxyphosphoryl)crotonate (8): Ethyl 4-bromocrotonate (20.00 g, 93.24 mmol) was added in one portion to triethyl phosphite (17.71 g, 106.56 mmol) at 120−130 °C and the solution was stirred for 1 h. Distillation of the resulting reaction mixture at 0.30 Torr provided **8** as a light yellow oil (20.39 g, 88 %). B.p. 115−118 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 6.66 (m, 1 H, 3-H), 5.75 (m, 1 H, 2-H), 3.93 (m, 6 H, CH_2CH$_3$), 2.56 (dd, ^2J = 22.9 Hz, ^3J = 7.8 Hz, 2 H, 4-H), 1.14 (m, 9 H, CH_3CH$_2$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 165.16 (s, C-1), 137.08 (d, ^2J = 12.6 Hz, C-3), 125.45 (d, ^3J = 10.5 Hz, C-2), 61.93 (*C*H$_2$CH$_3$), 61.81 (*C*H$_2$CH$_3$), 60.02 (*C*H$_2$CH$_3$), 30.20 (d, ^1J = 136.9 Hz, C-4), 16.06 (CH$_2$*C*H$_3$), 15.95 (CH$_2$*C*H$_3$), 13.81 (CH$_2$*C*H$_3$) ppm.

Ethyl (2*E*,4*E*)-6-Methylhepta-2,4-dienoate (9): Lithium bis(trimethylsilyl)amide (1.0 M in THF, 40 mL, 39.96 mmol) was added slowly to a solution of **8** (10.00 g, 39.96 mmol) in THF (18 mL) at −78 °C. The resulting dark orange solution was stirred at −78 °C for an additional 30 min before the dropwise addition of isobutyraldehyde (2.94 g, 39.96 mmol). The mixture was stirred at −78 °C for 1 h and then for 1 h at −40 °C, before being warmed to room temperature. The reaction was quenched with saturated aqueous ammonium chloride (90 mL) and extracted with diethyl ether (4 × 100 mL). The combined organic layers were washed with water (2 × 70 mL) and with brine (70 mL), dried with Na_2SO_4, filtered and concentrated in vacuo. Distillation of the crude residue at 1.1 Torr provided **9** (5.48 g, 82 %) as a colorless oil. B.p. 60−62 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.16 (dm, ^3J = 15.4 Hz, 1 H, 3-H), 6.00 (m, 2 H, 4-H and 5-H), 5.69 (d, ^3J = 15.4 Hz, 1 H, 2-H), 4.09 (q, $^3J_{a,b}$ = 7.1 Hz, 2 H, CH_2CH$_3$), 2.32 (m, 1 H, 6-H), 1.20 (t, ^3J = 7.1 Hz, 3 H, CH_3CH$_2$), 0.94 (d, ^3J = 6.8 Hz, 6 H, CH_3CHCH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 167.80 (C-1), 151.68 (C-4), 145.86 (C-3), 126.09 (C-5), 120.01 (C-2), 60.69 (*C*H$_2$CH$_3$), 32.10 (C-6), 22.37 (*C*H$_3$CH*C*H$_3$), 14.90 (*C*H$_3$CH$_2$) ppm. MS (ES, Na): *m*/*z* = 359.1 [2M + Na]$^+$, 191.0 [M + Na]$^+$.

(2*E*,4*E*)-6-Methylhepta-2,4-dienoic Acid (10): A solution of the ester **9** (4.10 g, 23.15 mmol) in MeOH (93 mL) was treated with a solution of KOH (3.67 g, 55.56 mmol) in water (15.5 mL). The resulting mixture was stirred for 48 h at ambient temperature and the MeOH was evaporated in vacuo. The crude residue was then treated with water and extracted with diethyl ether (3 × 50 mL). The resulting aqueous phase was adjusted to pH 1 treated with 2 M HCl and extracted again with diethyl ether (3 × 50 mL). The combined organic layers were dried with Na_2SO_4, filtered and concentrated to afford **10** (3.20 g, 98 %) as white crystals that were used without further purification. M.p. 35−37 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.33 (dm, $^3J_{3,2}$ = 15.1 Hz, 1 H, 3-H), 6.13 (m, 2 H, 4.5-H), 5.78 (d, $^3J_{2,3}$ = 15.1 Hz, 1 H, 2-H), 2.41 (m, 1 H, 6-H), 1.03 (d, ^3J = 6.8 Hz, 6 H, CH_3CHCH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.54 (C-1), 153.34 (C-4), 148.48 (C-3), 126.06 (C-5), 119.17 (C-2), 32.26 (C-6), 22.38 (*C*H$_3$CH*C*H$_3$) ppm. MS (ES, Na): *m*/*z* = 139.0 [M − H]$^-$, 140 [M$^+$].

Isooctanoic Acid (11): A solution of **9** (5.36 g, 31.86 mmol) in EtOAc (100 mL) was hydrogenated (4 atm) at room temperature in the presence of 5 % Pd/C (1.5 g). After 3.5 h, the palladium was removed by filtration and the filtrate evaporated to dryness under reduced pressure to afford the intermediate ester (5.28 g, 78 %) as a colorless oil that was used without further purification. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.08 (q, ^3J = 7.1 Hz, 2 H, CH$_3$CH_2), 2.24 (t, ^3J = 7.4 Hz, 2 H, 2-H), 1.48 (m, 3 H, 5-H and 6-H), 1.21 (t, ^3J = 7.1 Hz, 3 H, CH_3CH$_2$), 1.20 (m, 4 H, 3-H and 4-H), 0.82 (d, ^3J = 6.6 Hz, 6 H, CH_3CHCH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 173.82 (C-1), 60.10 (CH$_3$*C*H$_2$), 38.51 (C-2), 27.76 (C-6), 22.50 (*C*H$_3$CH*C*H$_3$), 14.19 (*C*H$_3$CH$_2$), [34.35, 26.89, 25.16 (C-3,4,5)] ppm.

A solution of this ester (5.40 g, 31.34 mmol) in absolute EtOH (50 mL) was treated with a solution of KOH (5.30 g, 94.02 mmol) in water (2 mL). The resulting solution was heated at reflux for 2 h before evaporation of the solvent in vacuo. The crude residue was then treated with water (5 mL), extracted with diethyl ether (3 × 30 mL) and the combined organic layers were washed with water (2 × 30 mL). The combined aqueous phases were adjusted to pH 1by treatment with 10 M hydrochloric acid and extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with water (2 × 50 mL) and with brine (50 mL), dried with Na_2SO_4, filtered and concentrated. Distillation of the crude residue at 18 Torr afforded **11** (3.1 g, 69 %) as a light yellow oil. B.p. 133−135 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.83 (d, ^3J = 6.5 Hz, 6 H, 7-H and 8-H), 1.24 (m, 4 H, 3-H and 4-H), 1.59 (m, 3 H, 5-H and 6-H), 2.32 (t, ^3J = 7.4 Hz, 2 H, 2-H), 11.55 (s, 1 H, OH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 180.60 (C-1), 38.54 (C-2), 27.79 (C-6), 22.54 (*C*H$_3$CH*C*H$_3$), [34.16, 26.87, 24.90 (C-3,4,5)] ppm.

(2*S*)-*N*-(6-Methylheptanoyl)serine (12): Ammonia was bubbled through a white suspension of L-serine methyl ester hydrochloride (1.32 g, 8.32 mmol) in CH_2Cl_2 (6.5 mL). After 5−10 min, the precipitate was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The residue was taken up in CH_2Cl_2 (6.5 mL) and treated with **5** (1.00 g, 6.93 mmol). The resulting solution was then cooled to −21 °C, treated with DCC (1.45 g, 6.93 mmol) and warmed to room temperature. After 12 h at ambient temperature, the white precipitate was removed by filtration and the filtrate concentrated. The residue was treated with diethyl ether (6 mL) and stirred at 0 °C for 2 h. This white precipitate was removed by filtration and the diethyl ether evaporated in vacuo. Flash column chromatography (SiO_2, EtOAc/heptane, 3:2) afforded the intermediate ester (1.43 g, 84 %) as a white solid. $[\alpha]_D^{20}$ = +19.5 (*c* = 1.17, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.47 (d, $^3J_{NH,2}$ = 7.1 Hz, 1 H, NH), 4.64 (ddd, $^3J_{2,NH}$ = 7.1 Hz, $^3J_{2,3a}$ = 4.0 Hz, $^3J_{2,3b}$ = 3.4 Hz, 1 H, 2-H), 3.94 (dd, $^3J_{3a,2}$ = 4.0 Hz, $^2J_{3a,3b}$ = 11.2 Hz, 1 H, 3-H^a), 3.75 (s, 3 H, CH$_3$O), 3.86 (dd, $^3J_{3b,2}$ = 3.4 Hz, $^2J_{3b,3a}$ = 11.2 Hz, 1 H, 3-H^b), 2.70 (s, 1 H, OH), 2.23 (t, $^3J_{2',3'}$ = 7.6 Hz, 2 H, 2′-H), 1.38 (m, 3 H, 3′-H and 6′-H), 1.38−1.28 (m, 2 H, 5′-H), 1.27−1.14 (m, 2 H, 4′-H), 0.82 (d, ^3J = 6.6 Hz, 6 H, CH_3CHCH_3), ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.91 (C-1), 172.16 (C-1′), 64.58 (CH$_3$O), 55.70 (C-3), 53.82 (C-2), 39.67 (C-5′), 37.59 (C-2′), 28.89 (C-6′), 28.05 (C-4′), 26.85 (C-3′), 23.63 (*C*H$_3$CH*C*H$_3$) ppm. MS (ES, Na): *m*/*z* = 268.1 [M + Na$^+$], 246 [M + H$^+$]. $C_{12}H_{23}NO_4$ (245.32): calcd. C 58.75, H 9.45, N 5.71; found C 58.75, H 9.28, N 5.62.

H_2O_2 (30 % wt in water, 250 μL) and aqueous LiOH (0.5 M, 20 mL, 10.19 mmol) were added dropwise to a solution of this ester (2.50 g, 10.19 mmol) in THF (25 mL). After addition, the THF was evaporated and the resulting aqueous solution extracted with diethyl ether (2 × 10 mL), diluted with EtOAc (10 mL) and adjusted to pH 1 by the slow addition of 1 M HCl. The resulting mixture was extracted with EtOAc (2 × 20 mL), the combined organic phases washed with brine, dried with $MgSO_4$, filtered and concentrated to afford **12** (2.00 g, 85 %) as a white solid that was used without further purification. $[\alpha]_D^{20} = +8.7$ (c = 1.08, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 4.53 (dd, $^3J_{2,3a}$ = 4.1 Hz, $^3J_{2,3b}$ = 5.0 Hz, 1 H, 2-H), 3.93 (dd, $^3J_{3b,2}$ = 5.0 Hz, $^2J_{3b,3a}$ = 11.2 Hz, 1 H, 3-H^b), 3.85 (dd, $^3J_{3a,2}$ = 4.1 Hz, $^2J_{3a,3b}$ = 11.2 Hz, 1 H, 3-H^a), 2.32 (t, $^3J_{2',3'}$ = 7.5 Hz, 2 H, 2′-H), 1.61 (m, 3 H, 3′-H and 6′-H), 1.39 (m, 2 H, 4′-H), 1.26 (m, 2 H, 5′-H), 0.93 (d, ^3J = 6.6 Hz, 6 H, CH_3CHCH_3) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 176.77 (C-1), 173.87 (C-1′), 63.36 (C-3), 56.44 (C-2), 40.28 (CH_2), 37.28 (C-2′), 29.45 (C-6′), 28.47 (CH_2), 27.49 (C-3′), 23.38 (CH_3CHCH_3) ppm.

(2*S*)-*N*-[(2*E*,4*E*)-6-Methylhepta-2,4-dienoyl]serine (13): Ammonia was bubbled through a white suspension of L-serine methyl ester hydrochloride (4.00 g, 25.2 mmol) in CH_2Cl_2 (20 mL). After 5−10 min, the resulting precipitate was removed by filtration and the filtrate was evaporated to dryness in vacuo. The residue was taken up in CH_2Cl_2 (50 mL) and treated with **10** (3.00 g, 21 mmol). The resulting solution was then cooled to −21 °C, treated with DCC (4.38 g, 21 mmol) and warmed to room temperature. After 12 h at ambient temperature, the white precipitate was removed by filtration and the filtrate was concentrated. The residue was treated with diethyl ether (25 mL) and stirred at 0 °C for 3 h. This white precipitate was removed by filtration and the diethyl ether was evaporated in vacuo. Flash column chromatography (SiO_2, EtOAc/cyclohexane, 3:7) afforded the coupled ester intermediate (3.83 g, 75 %) as a white solid. M.p. 35° −37 °C. $[\alpha]_D^{20} = +29.1$ (c = 1.02, CH_2Cl_2). ^{1}H NMR (300 MHz, CDCl3): δ = 7.18 (dm, $^3J_{3',2'}$ = 15.0 Hz, 1 H, 3′-H), 6.61 (d, ^3J = 7.3 Hz, 1 H, NH), 6.06 (m, 2 H, 4′-H and 5′-H), 5.85 (d, $^3J_{2',3'}$ = 15.0 Hz, 1 H, 2′-H), 4.72 (m, 1 H, 2-H), 3.94 (m, 2 H, 3-H), 3.76 (s, 3 H, CH_3O), 3.17 (s, 1 H, OH), 2.38 (m, 1 H, 6′-H), 1.0 (d, ^3J = 6.8 Hz, 6 H, CH_3CHCH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.80 (C-1′), 167.42 (C-1), 151.47 (C-4′), 143.51 (C-3′), 125.90 (C-5′), 121.55 (C-2′), 64.18 (C-3), 55.55 (C2), 53.44 (CH_3O), 32.13 (C-6′), 22.48 (CH_3CHCH_3) ppm. MS (ES, Na): m/z = 264.2 [M + Na]$^+$, 505.4 [2M + Na]$^+$. $C_{12}H_{19}NO_4$ (241.29): calcd. C 59.73, H 7.94, N 5.80, O 26.52; found C 59.77, H 8.22, N 5.78, O 26.47. Chiral HPLC (Analytical column: Chiralpak® AD; mobile phase: 30 % hexane and 70 % ethanol; flow rate = 0.9 mL/min; detection wavelength = 260 nm; room temperature, retention time = 15.2 min): *ee* ≥ 99 %.

H_2O_2 (30 % wt in water, 101 μL) and LiOH (0.5 M, 8.3 mL, 4.14 mmol) were added dropwise to a solution of the ester intermediate (1.00 g, 4.14 mmol) in THF (10 mL). After the addition, the THF was evaporated and water was added (10 mL). The resulting aqueous solution was extracted with diethyl ether (2 × 10 mL), diluted with EtOAc (10 mL) and adjusted to pH 1 by the slow addition of 1 M HCl. The resulting mixture was extracted with EtOAc, the combined organic phases washed with brine, dried with Na_2SO_4, filtered and concentrated to afford **13** (919 mg, 98 %) as a white solid that was used without further purification. M.p. 160 °C. $[\alpha]_D^{20} = +14.6$ (c = 1.02, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.09 (dd, $^3J_{3',4'}$ = 10.2 Hz, $^3J_{3',2'}$ = 15.1 Hz, 1 H, 3′-H), 6.09 (m, 2 H, 4′-H and 5′-H), 6.01 (d, $^3J_{2',3'}$ = 15.1 Hz, 1 H, 2′-H), 4.52 (dd, $^3J_{2,3a}$ = 4.9 Hz, $^3J_{2,3b}$ = 4.0 Hz, 1 H, 2-H), 3.87 (dd, $^3J_{3a,2}$ = 4.9 Hz, $^2J_{3a,3b}$ = 11.2 Hz, 1 H, 3-H^a), 3.78 (dd, $^3J_{3b,2}$ = 4.0 Hz, $^2J_{3b,3a}$ = 11.2 Hz, 1 H, 3-H^b), 2.36 (m, 1 H, 6′-H), 0.99 (d, ^3J = 6.8 Hz, 6 H, CH_3CHCH_3) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 173.51 (C-1′), 169.08 (C-1), 151.14 (C-4′), 143.22 (C-3′), 127.03 (C-5′), 122.89 (C-2′), 63.14 (C-3), 56.31 (C-3), 32.80 (C-6′), 22.38 (CH_3CHCH_3) ppm. MS (ES, Na): m/z = 226.1 [M − H]$^-$, 453.2 [2M − H]$^-$, 250.1 [M + Na]$^+$, 477.2 [2M + Na]$^+$. Chiral HPLC (Analytical column: Chiralpak® AD; mobile phase: 40 % hexane + 0.05 % TFA and 60 % ethanol + 0.05 % TFA; flow rate = 0.9 mL/min; detection wavelength = 260 nm; room temperature; retention time = 9.88 min): *ee* ≥ 99 %.

(1*R*,2*R*)-(−)-Pseudoephedrine Glycinamide (14): Triethylamine (25 mL, 0.178 mol) was added to a vigorously stirred solution of Boc-Gly-OH (25.98 g, 0.148 mol) in CH_2Cl_2 (500 mL) at 0 °C. After 5 min pivaloyl chloride (18.5 mL, 0.148 mol) was added dropwise. The resulting white slurry was stirred at 0 °C for 30 min before a second portion of triethylamine (25 mL, 0.178 mol) and (1*R*,2*R*)-pseudoephedrine (25.00 g, 0.148 mol) were successively and cautiously added (internal temperature < 3 °C !). After the addition, the resulting mixture was stirred at 0 °C for 40 min and concentrated in vacuo. The white residue was then dissolved in MeOH/water (1:1, 500 mL), cooled to 0 °C and cautiously treated with HCl (12 M, 195 mL) (internal temperature < 3 °C !). The resulting white precipitate was stirred for 3 h at 0 °C, warmed to room temperature and the MeOH was evaporated. The residue was then recooled to −20 °C and adjusted to pH 14 by the cautious addition of 50 % aqueous NaOH solution (internal temperature < 10 °C !). The solution was extracted with CH_2Cl_2 (5 × 300 mL), the combined organic layers dried with K_2CO_3, filtered through a short plug of Celite® and concentrated under reduced pressure. Crystallisation from toluene afforded **14** (22.86 g, 70 %) as tiny white crystals. M.p. 85−89 °C. $[\alpha]_D^{20} = -100.9$ (c = 1.11, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): 1:1 mixture of rotamers. δ = 7.24−7.36 (m, 5 H, Ar-H), 4.52−4.58 (m, 1 H, 2′-H), [4.47 (d, ^3J = 8.9 Hz, 0.5 H) + 3.77 (m, 0.5 H), 1′-H], [3.70 (d, ^3J = 15.9 Hz, 0.5 H) + 3.36 (d$_{obsd.}$, ^3J = 16.9 Hz, 1 H) + 3.27 (d, ^3J = 17.0 Hz, 0.5 H), 2-H], [2.90 (s, 1.5 H) + 2.74 (s, 1.5 H), NCH_3], 2.51 (s, 2 H, NH_2), [1.0 (d, ^3J = 6.5 Hz, 1.5 H) + 0.92 (d, ^3J = 6.8 Hz, 1.5 H), CH_3CH] ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 1:1 mixture of rotamers. δ = [174.95 + 174.04, C-1)], [142.80 + 142.48, (C$_{Ar}$C$_{2'}$)], [129.35 + 129.13, 128.90 + 128.53, 127.50 + 127.25, (C$_{Ar}$)], [76.70 + 75.75, (C-2′)], [58.32 + 58.20, (C-1′)], [44.42 + 44.14, (C-2)], [31.06 + 27.62, (CH_3N)], [16.09 + 14.98, (CH_3-C)] ppm. MS (ES, Na): m/z = 223.2 [M + H]$^+$, 245.1 [M + Na]$^+$, 467.3 [2M + Na]$^+$. $C_{12}H_{18}N_2O_2$ (222.29): calcd. C 64.84, H 8.16, N 12.60, O 14.40; found C 64.49, H 8.22, N 12.46, O 14.45.

(2*S*)-2-Amino-*N*-[(1*R*,2*R*)-2-hydroxy-1-methyl-2-phenylethyl]-*N*-methyl-4-methylpent-4-enamide (15): Anhydrous LiCl (10.87 g, 0.26 mol) was added to a solution of **14** (9.50 g, 42.74 mmol) in THF (240 mL). The mixture was cooled to −78 °C and treated with lithium bis(trimethylsilyl)amide (1.0 M in THF, 94.03 mL, 94.03 mmol). After the addition, the orange mixture was stirred at −78 °C for 1.5 h and at 0 °C for 1 h. 3-Bromo-2-methylpropene (4.89 mL, 47.01 mmol) was slowly added and the resulting yellow mixture was stirred at 0 °C for 15 min before the cautious addition of aqueous HCl (1 M, 294 mL) (internal temperature < 10 °C !). The light yellow solution was then washed with EtOAc (300 mL) and aqueous HCl (1 M, 295 mL) was added to the organic phase. The combined aqueous phases were cooled to −21 °C, adjusted to pH 14 by the cautious addition of 50 % aqueous NaOH (internal temperature < 0 °C !), and extracted with EtOAc (5 × 450 mL). The organic layers were dried with $MgSO_4$, filtered and concen-

trated. Flash column chromatography (SiO_2, CH_2Cl_2/MeOH/TEA, 92:4:4) afforded **15** (9.96 g, 84 %) as a viscous light yellow oil. $[\alpha]_D^{20} = -90.3$ (c = 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, CDCl3): 1:3 mixture of rotamers; resonances labelled with an asterisk * refer to the minor rotamer. δ = 7.30 (m, 5 H, ArH), 4.92 (s, 1 H, 5-H^a*), 4.86 (s, 1 H, 5-H^a), 4.83 (s, 1 H, 5-H^b*), 4.76 (s, 1 H, 5-H^b), 4.62 (s, 1 H, 2′-H*), 4.59 (s, 1 H, 2′-H), 4.47 (m, 1 H, 1′-H), 4.09 (m, 1 H, 1′-H*), 3.86 (m, 1 H, 2-H*), 3.76 (m, 1 H, 2-H), 2.94 (s, 3 H, NCH_3*), 2.87 (s, 3 H, NCH_3), 2.07 (m, 2 H, 3-H), 1.80 (s, 3 H, CH_3C=C*), 1.72 (s, 3 H, CH_3C=C), 1.07 (d, ^3J = 6.7 Hz, 3 H, CH_3CH), 0.97 (d, ^3J = 6.7 Hz, 3 H, CH_3CH*) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 1:3 mixture of rotamers; resonances labelled with an asterisk * refer to the minor rotamer. δ = 177.18 (C-1), 142.59 (C-4*), 141.75 (C-4), 129.18−126.86 (C-Ar), 114.30 (C-3), 76.37 (C-2′), 75.67 (C-2′*), 59.25 (C-1′), 58.31 (C-1′*), 50.43 (C-2), 50.16 (C-2*), 46.43 (C-5*), 44.58 (C-5), 32.71 (*C*H_3CH), 22.68 (*C*H_3C=C), 14.81 (*C*H_3N) ppm. MS (ES, Na): m/z = 277.2 [M + H]$^+$, 299.2 [M + Na]$^+$, 553.4 [2M + H]$^+$, 575.4 [2M + Na]$^+$.

(2*S*)-2-(*tert*-Butyloxycarbonylamino)-*N*-[(1*R*,2*R*)-2-hydroxy-1-methyl-2-phenylethyl]-*N*-methyl-4-methylpent-4-enamide (16): A solution of Na_2CO_3 (3.80 g, 35.46 mmol) in water (28 mL) was added to a solution of **15** (9.80 g, 35.46 mmol) in 1,4-dioxane (28 mL). The resulting mixture was cooled to 0 °C and treated with *tert*-butyldicarbonate (11.73 g, 53.19 mmol). After addition, the reaction mixture was stirred for 1 h at 0 °C, warmed to ambient temperature, stirred for an additional 1 h and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried with Na_2SO_4, filtered and concentrated under reduced pressure. The yellow residue was diluted with MeOH (140 mL) before the addition of K_2CO_3 (490 mg, 3.55 mmol). The resulting suspension was stirred at room temperature overnight. The MeOH was then evaporated in vacuo and the resulting gel was diluted with EtOAc/diethyl ether (1:1, 140 mL). The white needles were removed by filtration and the filtrate concentrated. Gradient flash chromatography (SiO_2, EtOAc/cyclohexane, 2:3, EtOAc/cyclohexane, 3:2) afforded **16** (10.29 g, 77 %) as a glassy solid. $[\alpha]_D^{20} = -67.9$ (c = 1.01, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): 1:3 mixture of rotamers; resonances labelled with an asterisk * refer to the minor rotamer. δ = 7.34−7.21 (m, 5 H, ArH), 5.22 (d, ^3J = 7.9 Hz, 1 H, OH), 5.13 (d, ^3J = 9.2 Hz, 1 H, OH*), 4.79 (s, 2 H, 5-H), 4.73 (s, 1 H, 5-H*), 4.64−4.55 (m, 2 H, 2-H and 2′-H), 4.50 (m, 1 H, 1′-H), 4.22 (m, 1 H, 1′-H*), 2.93 (s, 2 H, NCH_3), 2.91 (s, 1 H, NCH_3*), 2.60−2.11 (m, 2 H, 3-H*), 1.78 (s, 1 H, CH_3C=C*), 1.73 (s, 2 H, CH_3C=C), 1.39 (s, 9 H, Boc), 1.36* (s, 9 H, Boc*), 0.98−0.96 (m, 3 H, *C*H_3CH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 1:3 mixture of rotamers; resonances labelled with an asterisk * refer to the minor rotamer δ = 174.72 (C-1), 173.81 (C-1*), 156.90 (*C*OBoc*), 156.28 (*C*O-BOC), 148.38 (*C*$_{Ar}$-C$_{2'}$), 142.14 (*C*OBoc*), 142.01 (C-4*), 141.16 (C-4), [129.35 + 128.91 + 128.38 + 127.57 + 127.39, (C$_{Ar}$)], 114.88 (C-5), 114.32 (C-5*), 80.43 (*C*CH$_3$Boc), 79.92 (*C*CH$_3$Boc*), 76.29 (C-2), 75.97 (C-2*), 58.77 (C-1′), 50.13 (C-2′), 49.13 (C-2′*), 41.68 (C-3*), 41.44 (C-3), 32.46 (*C*H_3N), 28.90 (*C*H_3Boc), 27.82 (*C*H_3N*), 23.03 (*C*H_3C=C*), 22.94 (*C*H_3C=C), 16.19 (*C*H_3CH*), 14.93 (*C*H_3CH) ppm. HRMS (ES, Na): m/z = 399.22598 [M + Na]$^+$, calcd. for $C_{21}H_{32}N_2O_4$Na: m/z = 399.22598. $C_{21}H_{32}N_2O_4$ (376.49): calcd. C 66.99, H 8.57, N 7.44; found C 67.10, H 8.54, N 7.38.

(2*S*)-2-Amino-*N*-(*tert*-butyloxycarbonyl)-1-(2-furyl)-4-methylpent-4-en-1-one (17): *n*BuLi (8.71 mL, 13.94 mmol) was added dropwise to a solution of furan (1.05 mL, 14.34 mmol) in THF (11 mL) at −78 °C. After the addition, the colorless solution was warmed to 0 °C and stirred for 30 min. The resulting dark orange slurry was then recooled to −78 °C and a solution of **16** (1.50 g, 3.98 mmol) in THF (3.5 mL) was added slowly. This gave a dark yellow solution which was warmed to 0 °C and, after being stirred for 3 h, the dark orange solution was carefully poured into an mixture of ice and saturated aqueous NH_4Cl (350 mL) and extracted with diethyl ether (3 × 100 mL). The combined organic phases were washed with water (100 mL) and with brine (100 mL), dried with Na_2SO_4, filtered and concentrated in vacuo. Gradient flash column chromatography (SiO_2, EtOAc/cyclohexane, 5:95, EtOAc/cyclohexane, 1:9) followed by recrystallization from cyclohexane afforded **17** (883 mg, 80 %) as white crystals. M.p 74 °C. $[\alpha]_D^{20} = +74.3$ (c = 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.61 (d, $^3J_{5',4'}$ = 1.7 Hz, 1 H, 5′-H), 7.30 (d, $^3J_{3',4'}$ = 3.5 Hz, 1 H, 3′-H), 6.54 (dd, $^3J_{4',3'}$ = 3.5 Hz, $^3J_{4',5'}$ = 1.7 Hz, 1 H, 4′-H), 5.14 (s, 2 H, 2-H + NH), 4.81 (s, 1 H, 5-H^a), 4.72 (s, 1 H, 5-H^b), 2.55 (m, 1 H, 3-H^a), 2.27 (m, 1 H, 3-H^b), 1.76 (s, 3 H, CH_3C=C), 1.40 (s, 9 H, Boc) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.25 (C-1), 155.69 (C=O), 151.45 (C-2′), 147.45 (C-5′), 140.49 (C-4), 119.07 (C-3′), 114.98 (C-5), 112.93 (C-4′), 80.20 (*C*Boc), 54.29 (C-2), 41.59 (C-3), 28.68 (*C*H_3Boc), 22.52 (*C*H_3C=C) ppm. MS (ES, Na): m/z = 302.2 [M + Na]$^+$, 318.2 [M + K]$^+$, 581.3 [2M + Na]$^+$. $C_{15}H_{21}NO_4$ (279.33): calcd. C 64.50, H 7.58, N 5.01; found C 64.38, H 7.32, N 4.93. Chiral HPLC (4.6 × 250 mm analytical column, Daicel® OD; mobile phase: 90 % hexane and 10 % 2-propanol; flow rate = 1 mL/min; detection wavelength = 271 nm; room temperature; retention time = 6.12 min): *ee* = 96 %.

(2*S*)-2-Amino-1-(2-furyl)-4-methylpent-4-en-1-one Hydrochloride (19) and (18): A solution of HCl in 1,4-dioxane (4 M, 1.07 mL, 4.30 mmol) was added to **17** (100 mg, 0.36 mmol). The resulting purple mixture was stirred for 2 h at room temperature. The reaction mixture was then treated with diethyl ether and the mauve needles filtered to afford **19** (62 mg, 80 %). Evaporation of the filtrate afforded the cyclisation product **18** (20 %).

19: M.p 208 °C. ^{1}H NMR (300 MHz, DMSO): δ = 8.60 (s, 3 H, NH_3^+), 8.31 (d, $^3J_{5',4'}$ = 1.6 Hz, 1 H, 5′-H), 7.91 (d, $^3J_{3',4'}$ = 3.7 Hz, 1 H, 3′-H), 6.98 (dd, $^3J_{4',5'}$ = 1.6 Hz, $^3J_{4',3'}$ = 3.7 Hz, 1 H, 4′-H), 4.99 (s, 1 H, 5-H^b), 4.98 (m, 1 H, 2-H), 4.94 (s, 1 H, 5-H^a), 2.60−2.75 (m, 2 H, 3-H), 1.89 (s, 3 H, CH_3C=C) ppm. ^{13}C NMR (75 MHz, DMSO): δ = 183.33 (C-1), 149.69 (C-5′ and C-2′), 138.86 (C-4), 121.97 (C-3′), 116.38 (C-5), 113.61 (C-4′), 52.65 (C-2), 39.60 (C-3), 22.23 (*C*H_3C=C) ppm. MS (ES, Na): m/z = 180.2 [M − Cl]$^-$. Chiral HPLC (4.6 × 250 mm column, Daicel® OD; mobile phase: 85 % hexane + 0.1 % TEA and 15 % 2-propanol + 0.1 % TEA; flow rate = 1 mL/min; detection wavelength = 270 nm; room temperature; retention time = 7.49 min): *ee* = 96 %. **18:** ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.61 (d, $^3J_{5',4'}$ = 1.7 Hz, 1 H, 5′-H), 7.35 (d, $^3J_{3',4'}$ = 3.6 Hz, 1 H, 3′-H), 6.60 (dd, $^3J_{4',5'}$ = 1.7 Hz, $^3J_{4',3'}$ = 3.6 Hz, 1 H, 4′-H), 5.79 (s, 1 H, NH), 4.82 (dd, $^3J_{4,5a}$ = 4.7 Hz, $^3J_{4,5b}$ = 11.9 Hz, 1 H, 4-H), 2.44 (dd, $^3J_{5a,4}$ = 4.7 Hz, $^2J_{5a,5b}$ = 13.5 Hz, 1 H, 5-H^a), 1.79 (dd, $^3J_{5b,4}$ = 11.9 Hz, $^2J_{5b,5a}$ = 13.5 Hz, 1 H, 5-H^b), 1.53 (s, 3 H, CH_3CCH$_3$), 1.45 (s, 3 H, CH$_3$C*C*H$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 185.11 (C-1′), 152.20 (C-2), 147.59 (C-5′), 145.51 (C-2′), 119.88 (C-3′), 113.70 (C-4′), 79.26 (C-6), 54.89 (C-4), 36.23 (C-5), [30.00 + 26.03, (*C*H_3C*C*H$_3$)] ppm. MS (ES, Na): m/z = 246.1 [M + Na]$^+$, 469.3 [2M + Na]$^+$.

(*S*,*S*)-Compound 4: PyBOP (93.83 mg, 0.18 mmol), HOBt (21.92 mg, 0.16 mmol) and acid **12** (41.29 mg, 0.18 mmol) were added successively to a suspension of **19** (35 mg, 0.16 mmol) in DMF (5 mL). The resulting light mauve mixture was cooled to 0 °C and treated with NEM (20.86 μL, 0.16 mmol). The reaction mixture was stirred for 1 h at 0 °C followed by 30 min at room temperature

before being recooled to 0 °C and quenched with brine (30 mL). The resulting mixture was extracted twice with diethyl ether (10 mL) and twice with EtOAc (10 mL). The combined organic layers were then successively washed with 2 % aqueous $KHSO_4$, with saturated aqueous $NaHCO_3$ and with brine, dried with Na_2SO_4, filtered and concentrated in vacuo. Gradient flash column chromatography (SiO_2, EtOAc/cyclohexane, 2:3, EtOAc/cyclohexane, 1:1) afforded **4** (35 mg, 55 %) as a glassy solid. $[\alpha]_D^{20} = +11.3$ (c = 1.15, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.63 (dd, $^3J_{5''',3'''}$ = 0.6 Hz, $^3J_{5''',4'''}$ = 1.7 Hz, 1 H, 5′′′-H), 7.33 (dd, $^3J_{3''',5'''}$ = 0.6 Hz, $^3J_{3''',4'''}$ = 3.6 Hz, 1 H, 3′′′-H), 7.14 (d, ^3J = 7.5 Hz, 1 H, NH), 6.57 (dd, $^3J_{4''',5'''}$ = 1.7 Hz, $^3J_{4''',3'''}$ = 3.6 Hz, 1 H, 4′′′-H), 6.51 (d, ^3J = 7.1 Hz, 1 H, NH), 5.37 (m, 1 H, 1′′-H), 4.82 (s, 1 H, 4′′-H^a), 4.74 (s, 1 H, 4′′-H^b), 4.51 (m, 1 H, 1′-H), 4.00 (dd, $^3J_{2'a,1'}$ = 3.6 Hz, $^2J_{2'a,2'b}$ = 11.4 Hz, 1 H, 2′-H^a), 3.57 (dd, $^3J_{2'b,1'}$ = 5.6 Hz, $^2J_{2'b,2'a}$ = 11.4 Hz, 1 H, 2′-H^b), 3.56 (s, 1 H, OH), 2.59 (dd, $^3J_{2''a,1''}$ = 4.3 Hz, $^2J_{2''a,2''b}$ = 14.2 Hz, 1 H, 2′′-H^a), 2.35 (dd, $^3J_{2''b,1''}$ = 9.57 Hz, $^2J_{2''b,2''a}$ = 14.2 Hz, 1 H, 2′′-H^b), 2.20 (t, $^3J_{2,3}$ = 7.7 Hz, 2 H, 2-H), 1.75 (s, 3 H, 5-H), 1.51 (m, 3 H, 3-H and 6-H), 1.18 (m, 2 H, 4-H), 1.15 (m, 2 H, 5-H), 0.83 (d, ^3J = 6.6 Hz, 6 H, CH_3CHCH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 187.31 (C=OC$_{2'''}$), 174.16 (C=ONH), 171.36 (C-1), 151.30 (C-2′′′), 147.62 (C-5′′′), 140.50 (C-3′′), 119.39 (C-3′′′), 115.20 (C-4′′), 113.14 (C-4′′′), 63.35 (C-2′), 54.00 (C-1′), 53.58 (C-1′′), 40.69 (C-2′′), 38.97 (C-4 or C-5), 36.89 (C-2), 28.18 (C-6), 27.41 (C-4 or C-5), 26.17 (C-3), 22.96 (CH$_3$CHCH$_3$), 22.33 (C-5′′) ppm. MS (ES, Na): m/z = 393.2 [M + H]$^+$, 415.2 [M + Na]$^+$, 431.2 [M + K]$^+$. HRMS (ES, Na): m/z = 415.22089 [M + Na]$^+$, calcd. for $C_{21}H_{32}N_2O_5Na$: m/z = 415.22089. $C_{21}H_{32}N_2O_5$ (392.49): calcd. C 64.26, H 8.22, N 7.14, O 20.38; found C 64.03, H 8.44, N 7.17, O 20.43. Chiral HPLC (3 × 100 mm analytical column, Waters® X-Terra, mobile phase: 72 % water and 28 % acetonitrile; flow rate = 0.8 mL/min; detection wavelength = 280 nm; room temperature; retention time = 20.73 min): *de* = 96 %.

(*S*,*S*,*E*)-Compound 5: PyBOP (265.1 mg, 0.51 mmol), HOBt (42.56 mg, 0.32 mmol) and acid **13** (98.1 mg, 0.41 mmol) were added successively to a suspension of **19** (68 mg, 0.32 mmol) in DMF (5 mL). The resulting dark orange mixture was cooled to 0 °C and treated with NEM (40.4 μL, 0.32 mmol). The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 30 min before being recooled to 0 °C, at which point is was quenched with brine (60 mL). The resulting mixture was extracted twice with diethyl ether (20 mL) and twice with EtOAc (20 mL). The combined organic layers were then washed successively with 2 % aqueous $KHSO_4$, saturated aqueous $NaHCO_3$ and brine, dried with Na_2SO_4, filtered and concentrated in vacuo. Gradient flash column chromatography (SiO_2, EtOAc/cyclohexane, 2:3, EtOAc/cyclohexane, 1:1) afforded **5** (66 mg, 54 %) as a glassy solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.63 (d, $^3J_{5''',4'''}$= 1.7 Hz, 1 H, 5′′′-H), 7.33 (d, $^3J_{3''',4'''}$ = 3.6 Hz, 1 H, 3′′′-H), 7.24 (m, 1 H, 3-H), 7.13 (d, ^3J = 7.6 Hz, 1 H, NH), 6.57 (dd, $^3J_{4''',5'''}$ = 1.7 Hz, $^3J_{4''',3'''}$ = 3.6 Hz, 1 H, 4′′′-H), 6.46 (d, ^3J = 6.4 Hz, 1 H, NH), 6.08 (m, 2 H, 4.5-H), 5.79 (d, $^3J_{2,3}$ = 14.9 Hz, 1 H, 2-H), 5.37 (m, 1 H, 1′′-H), 4.80 (s, 1 H, 4′′H^a), 4.71 (s, 1 H, 4′′-H^b), 4.57 (m, 1 H, 1′-H), 4.11 (m, 1 H, 2′-H^a), 3.46 (m, 1 H, 2′-H^b), 3.38 (sl, 1 H, OH), 2.61 (dd, $^3J_{2''a,1''}$ = 4.3 Hz, $^2J_{2''a,2''b}$ = 14.0 Hz, 1 H, 2′′-H^a), 2.33 (m, 2 H, 6-H and 2′′-H^b), 1.74 (s, 3 H, 5′′-H), 1.02 (d, ^3J = 6.7 Hz, 6 H, CH_3CHCH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 187.60 (C=OC$_{2'''}$), 171.61 (C-1), 167.52 (C=ONH), 151.55 (C-5), 151.37 (C-2′′′), 147.90 (C-5′′′), 143.47 (C-3), 140.71 (C-3′′), 125.97 (C-4), 121.56 (C-2), 119.71 (C-3′′′), 115.52 (C-4′′), 113.38 (C-4′′′), 63.61 (C-2′), 54.57 (C-1′), 53.85 (C-1′′), 40.99 (C-2′′), 32.14 (C-6), 22.63 (C-5′′), 22.48 (CH$_3$CHCH$_3$) ppm. MS (ES, Na): m/z = 389.2 [M + H]$^+$, 411.2 [M + Na]$^+$, 427.2 [M + K]$^+$, 799.4 [2M + Na]$^+$. HRMS (ES, Na): m/z = 411.18959 [M + Na]$^+$, calcd. for $C_{21}H_{28}N_2O_5Na$: m/z = 411.18959. $C_{21}H_{28}N_2O_5$ (338.46): calcd. C 64.93, H 7.27, N 7.21, O 20.59; found C 64.86, H 7.57, N 6.97, O 20.24. Chiral HPLC (3 × 100 mm analytical column, Waters® X-Terra; mobile phase: 75 % water and 25 % acetonitrile; flow rate = 0.8 mL/min; detection, 270 nm; temperature, room temperature; retention time = 26.61−30.32 min): *de* = 48 %.

***N*-[(2*E*,4*E*)-6-Methylhepta-2,4-dienoyl][(2*S*)-seryl]glycine Methyl Ester:** PyBOP (347 mg, 0.66 mmol), HOBt (86.1 mg, 0.60 mmol) and acid 13 (150 mg, 0.66 mmol) were added successively to a suspension of glycine methyl ester hydrochloride (76.1 mg, 0.60 mmol) in DMF (7 mL). The resulting mixture was cooled to 0 °C and treated with NEM (77.1 μL, 0.60 mmol). The reaction mixture was stirred for 1 h at 0 °C and for 2 h at room temperature before being recooled to 0 °C and quenched with brine (60 mL). The resulting mixture was extracted twice with diethyl ether (20 mL) and twice with EtOAc (20 mL). The combined organic layers were then washed successively with 2 % aqueous $KHSO_4$, saturated aqueous $NaHCO_3$ and brine, dried with Na_2SO_4, filtered and concentrated in vacuo. Flash column chromatography (SiO_2, EtOAc/cyclohexane, 7:3) afforded the coupling product (100 mg, 51 %) as a light orange oil. ^{1}H NMR (300 MHz, CDCl3): δ = 7.39 (s, 1 H, NH), 7.20 (m, 1 H, 3′′-H), 6.74 (d, ^3J = 7.2 Hz, 1 H, NH), 6.12−6.09 (m, 2 H, 4′′-H and 5′′-H), 5.87 (d, $^3J_{2'',3''}$ = 15.0 Hz, 1 H, 2′′-H), 4.60 (m, 1 H, 2-′H), 4.17 (dd, $^3J_{3'a,2'}$ = 3.1 Hz, $^2J_{3'a,3'b}$ = 11.6 Hz, 1 H, 3′-H^a), 4.04 (d, ^3J = 5.7 Hz, 2 H, 2-H), 3.75 (s, 3 H, CH_3O), 3.70 (dd, $^3J_{3'b,2'}$ = 4.9 Hz, $^2J_{3'b,3'a}$ = 11.6 Hz, 1 H, 3′-H^b), 3.14 (s, 1 H, OH), 2.42 (m, 1 H, 6′′-H), 1.04 (d, ^3J = 6.8 Hz, 6 H, CH_3CHCH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.33 (C-1′), 170.77 (C-1′′), 167.95 (C-1), 151.91 (C-5′′), 143.97 (C-3′′), 125.89 (C-4′′), 121.14 (C-2′′), 63.42 (C-3′), 54.62 (C-2′), 53.19 (CH$_3$O), 41.89 (C-2), 32.2 (C-6′′), 22.49 (CH$_3$CHCH$_3$) ppm. MS (ES, Na): m/z = 321.3 [M + Na]$^+$, 322.3 [MH + Na]$^+$, 337.3 [M + K]$^+$. HRMS (ES, Na): m/z = 321.14264 [M + Na]$^+$, calcd. for $C_{14}H_{22}N_2O_5Na$: m/z = 321.14264. $C_{14}H_{22}N_2O_5$ (298.34): calcd. C 56.36, H 7.43, N 9.39, O 26.81; found C 56.25, H 7.42, N 9.69, O 26.64. Chiral HPLC (analytical column, Chiralpak® AD; mobile phase: 60 % hexane and 40 % 2-propanol; flow rate = 1 mL/min; detection wavelength = 259 nm; room temperature; retention time = 7.69−14.72 min): *de* = 58 %.

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