Application of acyl cyanophosphorane methodology to the synthesis of protease inhibitors: poststatin, eurystatin, phebestin, probestin and bestatin

Harry H. Wasserman,* Anders K. Petersen and Mingde Xia

Department of Chemistry, Yale University, P.O. Box 208107, New Haven, CT 06520-8107 USA

Abstract—Full details are given for the syntheses of the protease inhibitors, poststatin and eurystatin by the acyl cyanophosphorane coupling procedure used for the formation of α-keto amides. We have also extended this methodology to the syntheses of the related α-hydroxy amide natural products, phebestin, probestin and bestatin. The key step in the latter synthetic sequences involved diastereomeric selectivity in the reduction of the α-keto precursor to the corresponding α-hydroxy amide by the use of zinc borohydride.
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1. Introduction

In the course of an extensive investigation of the chemistry of vicinal tricarbonyls, we prepared a series of peptidyl tricarbonyl derivatives which showed notable activity as serine protease inhibitors. These tricarbonyl aggregates were formed from orthogonally protected peptidic carboxylic acids by coupling with benzyl(triphenylphosphoranylidene)acetate in the presence of EDCI. The peptidyl keto ylide esters thus formed were subsequently oxidized by Oxone®, ozone or DMD to the corresponding tricarbonyl esters (Scheme 1).[1]

More recently, we have modified this carbonyl-insertion protocol using (cyanomethylene)triphenylphosphorane (**2**) in the formation of stable acyl cyanophosphorane intermediates **3** from carboxylic acids **1**. The products were then converted by ozone at low temperature to labile α,β-diketo nitriles. These powerful electrophiles undergo rapid reaction with nucleophiles such as alcohols and amines to yield α-keto esters (**5a**) or amides (**5b**), respectively (Scheme 2).[2,3]

The present work describes the use of this acyl cyano-phosphorane procedure in the formation of a series of natural products containing α-keto or α-hydroxy amide linkages which are of special interest as protease inhibitors.[4–14] The synthetic targets included poststatin and eurystatin, inhibitors of prolyl endopeptidases. The methodology was also applied to the preparation of the aminopeptidase inhibitors, phebestin, probestin and bestatin by stereocontrolled reduction of initially formed α-keto amides to the corresponding α-hydroxy amides.

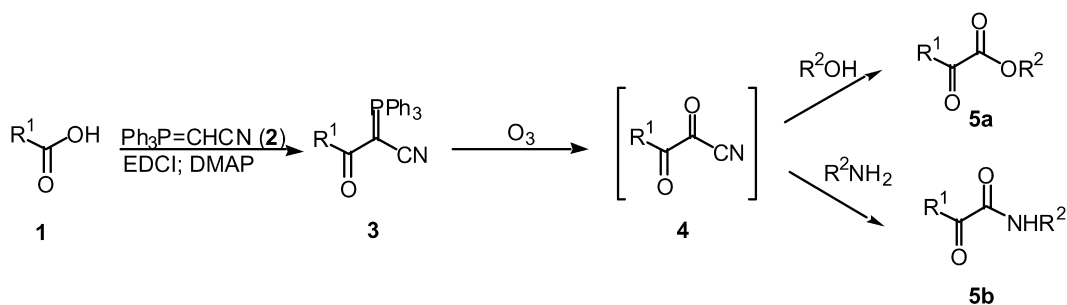## 2. Results and discussion

2.1. Poststatin (6)

Poststatin (**6**) is a naturally-occurring pentapeptide isolated from *Streptomyces viridochromogenes*. The sequence is H-Val-Val-Pos-D-Leu-Val-OH,[15] where Pos is the unusual (*S*)-3-amino-2-oxopentanoic acid named L-postine. The α-keto amide group appears to be essential for the biological
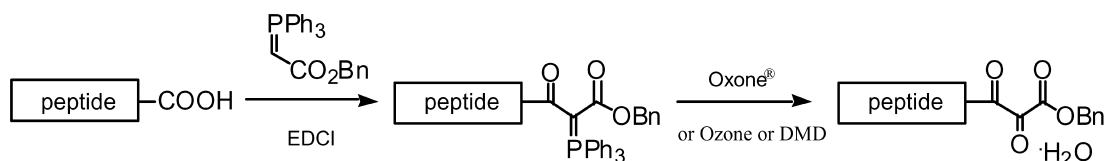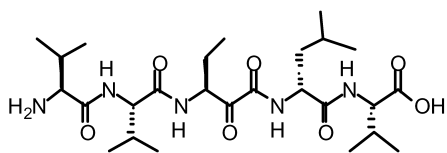

Scheme 1.

Scheme 2.

activity of this oligopeptide as is the case for a variety of cyclic peptides, such as the cyclotheonamides,[5a] orbiculamides[6] and eurystatins,[9] among other related bioactive products.


Poststatin (6)

An earlier synthesis of poststatin followed an established route[15d,f,16] for the formation of enzyme inhibitors in this family, involving formation of α-hydroxy amide precursors, which could then be oxidized to the final ketone-derived products. The present procedure which has general applicability and requires fewer steps follows the plan outlined in Scheme 3.[17]

Two pathways were originally considered for assembling poststatin (6) which can be considered to be a postine residue substituted at both ends by a dipeptide fragment. In one route, the N-terminal would be coupled to *N*-protected valylvaline, followed by a second-stage C-terminal reaction with *O*-protected D-leucylvaline. In a second approach, the order of coupling would be reversed. Our choice of the first route (Scheme 3) was based on preference for a pathway which would postpone the ozone oxidation of the acyl cyanophosphorane intermediate to the penultimate step, thereby minimizing any possible epimerization of the postine residue.

The synthesis started with the acyl cyanophosphorane **8**, formed (88%) by the EDCI coupling of commercially available Cbz-protected (*S*)-(+)-2-aminobutanoic acid **7** with (cyanomethylene)triphenylphosphorane (**2**). After removal of the Cbz-protecting group by hydrogenolysis,[18] the amine **9** was treated with Cbz-protected valylvaline **10** under standard peptide-coupling conditions yielding the tripeptide **11** as the sole epimer. Ozonolytic cleavage of the
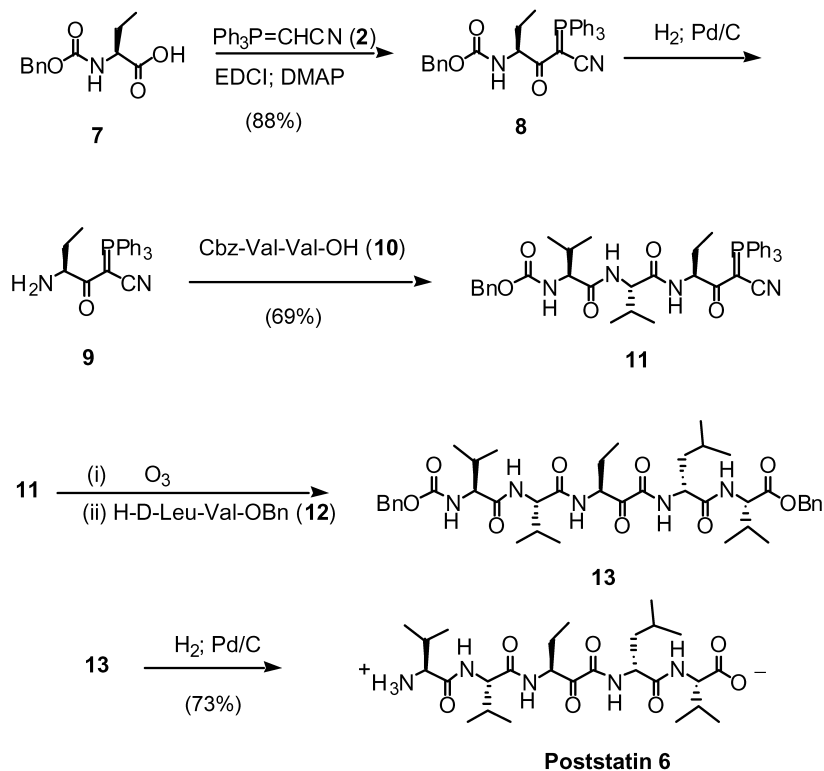
Scheme 3.
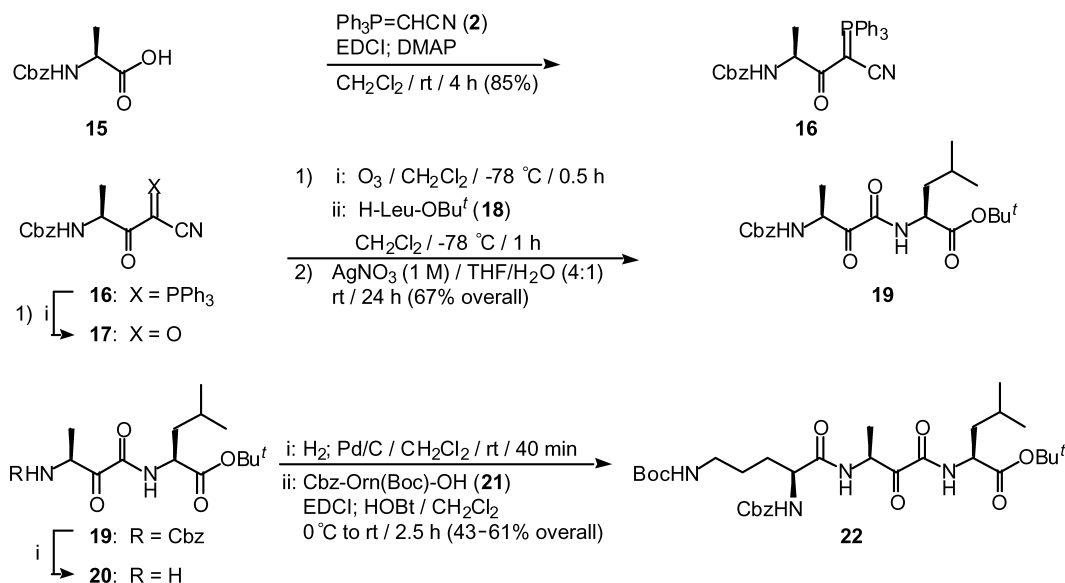
Scheme 4.

carbon–phosphorus double bond in **11** generated a labile α,β-diketo nitrile intermediate which was allowed to react in situ with D-leucylvaline *O*-benzyl ester **12** to form the protected pentapeptide **13**. Essentially no epimerization of the postine unit was observed at this stage.

Hydrogenolysis of the benzylic protecting groups of **13** yielded crude product **6**. NMR examination suggested the presence of 15–20% of a poststatin epimer. Purification by reverse-phase chromatography yielded the desired product, which was identical in all respects to an authentic sample of poststatin (**6**).[19]
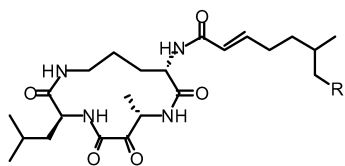
2.2. Eurystatin A (14a)

Eurystatins A and B (**14a** and **b**), inhibitors of prolyl endopeptidase, isolated from *Streptomyces eurythermus*, incorporate (*S*)-3-amino-2-oxobutanoic acid, leucine and ornithine in a 13-membered ring.[20] Like the cyclotheona-

mide thrombin inhibitors and the PED inhibitor, poststatin, they contain an α-keto amide residue which is thought to play a significant role in the enzyme inhibition.[5] A previous synthesis of **14a** featured the formation of an α-hydroxy amide precursor which was oxidized to the α-keto grouping at a late stage of the procedure.[15a,b,e]

Two routes were investigated for the synthesis of the eurystatins, both of which generated the tripeptide **22** as a key intermediate.[21] The first synthesis (Scheme 4) began with Cbz-protected alanine **15**, which was converted to the acyl cyanophosphorane **16** with (cyano-methylene)triphenylphosphorane (**2**). Ozonolysis of **16** generated the corresponding diketo nitrile **17**, which was reacted in situ with leucine *tert*-butyl ester **18** to give the dipeptide **19**. Removal of the Cbz-protecting group from **19** yielded the α-amino vicinal dicarbonyl product **20**. Coupling with the carboxyl group of di-*N*-protected ornithine **21** yielded the carbonyl-extended tripeptide **22**.
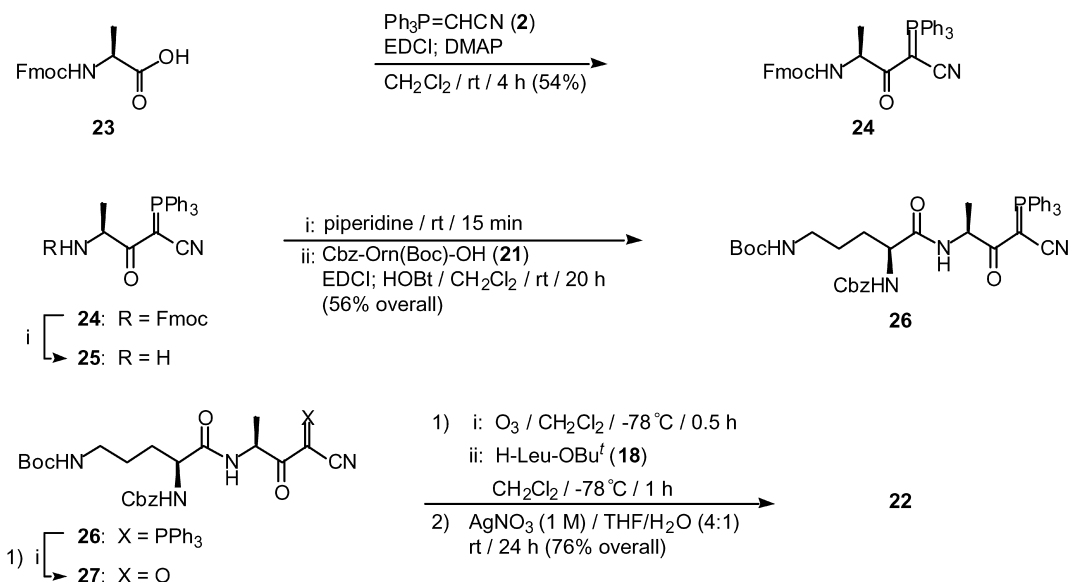
Scheme 5.

R = H, **Eurystatin A (14a)**
R = Me, **Eurystatin B (14b)**

In the second route (Scheme 5), featuring an ylide-stabilized α-amino ketone **25**, the ozonolysis was carried out at a later stage in the synthesis. The required Fmoc-protected acyl cyanophosphorane **24** was prepared from Fmoc alanine (**23**) by our general coupling procedure.

The Fmoc protecting group was eliminated smoothly with piperidine, and the crude amine **25** was coupled with the ornithine derivative **21** affording the cyanophosphorane **26**. Ozonolysis yielded **27**, which was trapped with leucine *tert*-butyl ester (**18**) to form the tripeptide **22**.

Of the two procedures for the preparation of **22**, the route given in Scheme 5 is preferred, since as in the poststatin synthesis,[17] the deferral of the ozonolysis until a late stage in the sequence provides better protection for the vicinal dicarbonyl system and the chiral centers during the deprotection steps.

Simultaneous removal of the protecting groups at the δ-amino and the carboxyl termini of tripeptide **22** to form **28** was accomplished with TFA (Scheme 6). Cyclization of **28** to **29** was performed in DMF under conditions of high dilution using diphenyl phosphorylazide (DPPA) and sodium hydrogen carbonate. Following hydrogenolysis of the cyclic tripeptide **29**, the amine **30** was coupled with (*E*)-6-methyl-2-hepteneoic acid **31**[22] to yield eurystatin A (**14a**) identical to the natural material.[23,24]

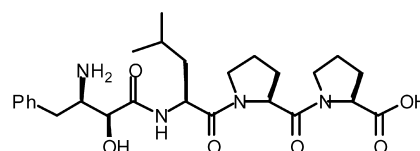### 2.3. α-Keto amides in the synthesis of hydroxy peptides

We next directed our attention to a group of hydroxy peptides containing a β-amino α-hydroxy amide residue exemplified by the natural products phebestin, probestin and bestatin.
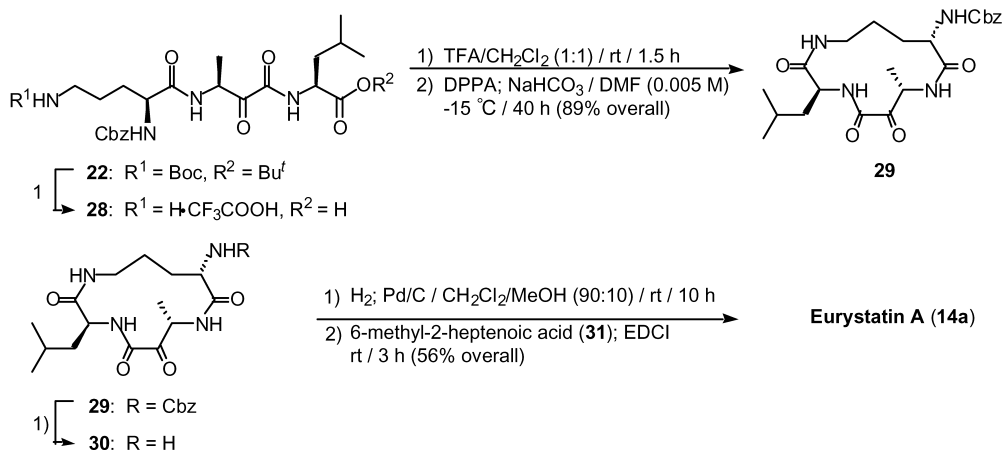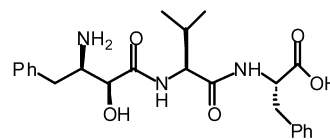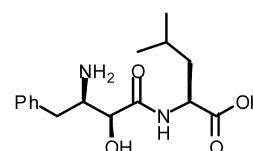

Phebestin (37)

Probestin (41)

Bestatin (42)

These products have been the object of studies leading to the development of several synthetic approaches, including Sharpless's asymmetric aminohydroxylation of α,β-unsaturated amides, Ojima's ring-opening of β-lactams and the asymmetric catalytic reduction of α-keto carboxylic acids.[25] Our success in preparing α-keto amides from carboxylic acids by the facile procedure outlined in Scheme 2 prompted us to explore the diastereoselective reduction of these keto derivatives as a simple, direct route to the α-hydroxy amide system.

2.4. Phebestin

In the synthesis of phebestin, outlined in Scheme 7, it was found that the best results were obtained by carrying out the reduction at an early stage of the assembly of the tripeptide framework. Accordingly, the synthesis began with *N*-Boc-D-phenylalanine (**32**) which was coupled with (cyano-methylene)triphenylphosphorane (**2**) to form the acyl cyanophosphorane **33**. Ozonolysis of **33** was followed by
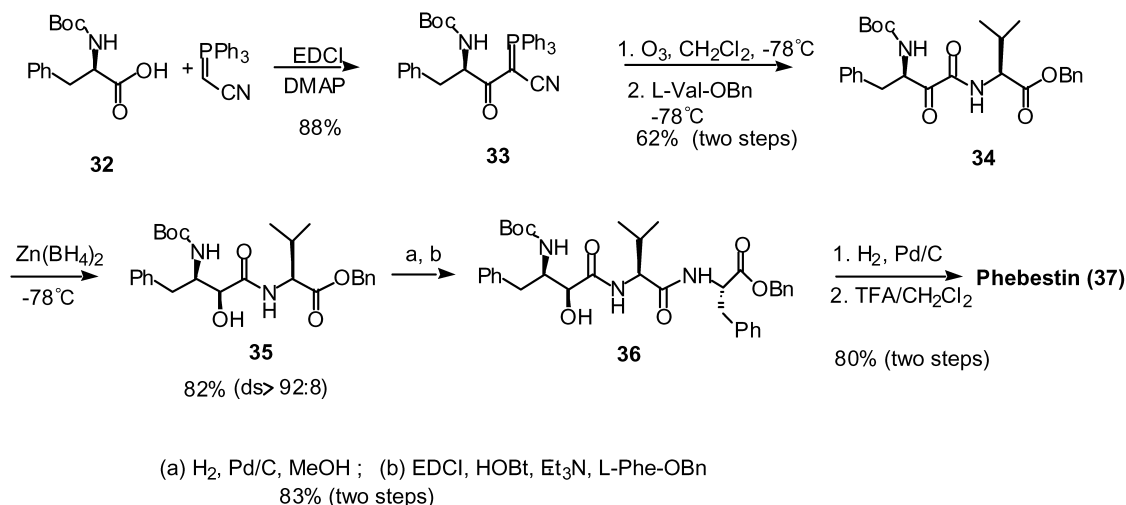
Scheme 6.

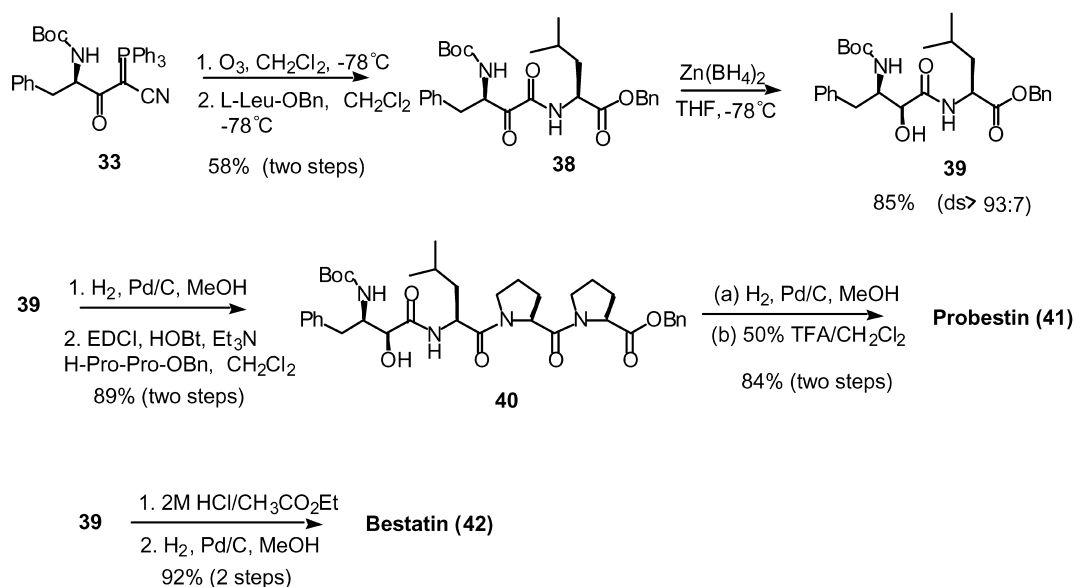**Scheme 7.**

coupling with the benzyl ester of L-valine at low temperature to yield the α-keto amide **34**. At this early point, we explored the use of reducing agents for the diastereoselective conversion of **34** to **35**.

Among the reducing agents which were examined,[25] including complexes of rhodium with chiral ligands, Dip-Cl, DIBAL-H, zinc borohydride, L-Selectride®, K-selectride® and (Bu^tO)$_3$BH–Li, it was found that zinc borohydride resulted in the highest diastereomeric selectivity (92:8) in the reduction of the keto substrate to the alcohol. In practice, the borohydride (0.15 M) in ether (2 equiv.) was added with stirring to the solution of the α-keto amide **34** in THF at −78°C under N$_2$. After separation of the desired diastereomer **35** by preparative TLC, the product was deprotected by hydrogenolysis and then coupled with the benzyl ester of L-phenylalanine to yield the tripeptide **36**. Removal of protecting groups from **36**, using hydrogenolysis and TFA sequentially, yielded a product **37** (80%) identical in every respect with natural

phebestin.[26] The selectivity in the reduction of **34** to **35** may be explained on the basis of chelation control in which the two carbonyl groups coordinate with the zinc ion permitting hydride attack at the less hindered side of the α-carbonyl group.

2.5. Probestin and bestatin

In the synthesis of probestin, Scheme 8, the unusual β-amino-α-hydroxy amide linkage was introduced at the dipeptide stage for further coupling with a second dipeptide unit. Thus, the doubly protected dipeptide **38**, containing the α-keto amide residue, was subjected to reduction by zinc borohydride to give the α-hydroxy product **39** with diastereomeric selectivity 93:7 (85%). Further transformation of **39**, by hydrogenolysis to remove the benzyl group followed by coupling with the benzyl ester of prolylproline, gave **40**. This was followed by sequential deprotection with hydrogenation and TFA to yield probestin (**41**).

Scheme 8.

In an accompanying study during the probestin synthesis, the β-amino-α-hydroxy intermediate **39** was deprotected. The product was shown to be identical in every respect to an authentic sample (Sigma) of bestatin (**42**).

3. Experimental

3.1. General methods

All reactions were carried out in oven-dried glassware under an atmosphere of N_2. Solvents and reagent solutions were transferred using gastight syringes. Tetrahydrofuran (THF), benzene (PhH), and dichloromethane (CH_2Cl_2) were distilled from calcium hydride (CaH_2) under dinitrogen. Other solvents (A.C.S. spectrophotometric grade) were dried over 3-Å molecular sieves, when necessary, and used without further purification. Commercially available reagents were purchased from Aldrich Chemical Company, Inc., Wisconsin. Ozone was generated from dry dioxygen using a Welsbach ozonator.

Flash chromatography was conducted on silica gel (40–63 μm, Merck Silica Gel 60) by the method of Still et al.[27] All chromatographic purification was monitored by thin-layer chromatography (TLC) on 250 μm 60 F_{254} silica-gel plates from Merck. The compounds were visualized under ultraviolet light (UV), iodine (I_2), and/or by heating with phosphomolybdic acid stain (PMA): 5% PMA in ethanol. Melting intervals were determined with a Thomas–Hoover capillary melting point apparatus. Optical rotations ($[\alpha]_D^{20}$) were determined on a Perkin–Elmer 241 polarimeter. Infrared spectra (IR) were measured in chloroform ($CHCl_3$) using a NaCl cuvette on a Perkin–Elmer IR 1420 spectrophotometer. ^{1}H NMR data were obtained using a Bruker AM-500 spectrometer (500 MHz) with tetramethyl-silane (TMS, $\delta \equiv 0$) as internal standard, or (methyl sulfoxide)-d_6 (DMSO-d_6, $\delta = 2.49$ ppm for residual ^{1}H). Proton-decoupled ^{13}C NMR spectra were recorded in deuteriochloroform (CDCl$_3$, $\delta = 77.0$ ppm), deuterio-methylene chloride (CD$_2$Cl$_2$, $\delta = 53.8$ ppm), or (methyl sulfoxide)-d_6 (DMSO-d_6, $\delta = 39.5$ ppm) on the same instrument at 125 MHz. Data are reported as follows: chemical shift in parts per million downfield from TMS (δ), multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, quint=quintet, m=multiplet, (br=broad)), coupling constants (J or $\langle J \rangle$ for an average value) in Hz, integration, and assignments. Low-resolution mass spectra (MS) and high-resolution mass spectra (HRMS) were done at the Mass Spectrometry Facility at the Yale Medical School, Yale University. Exact molecular masses are given for the isotopes ^{1}H, ^{12}C, ^{14}N, ^{16}O, ^{19}F, [31]P. Elemental analyses (EA) were obtained from Atlantic Microlab, Inc., Georgia.

3.2. Experimental procedures used in the synthesis of poststatin

3.2.1. [(3-Cyano-1-ethyl-2-oxo-3-(triphenyl-λ⁵-phos-phanylidene)-propyl]-carbamic acid benzyl ester (8). Cbz-Abu-OH (**7**) (9.071 g, 38.23 mmol) was dissolved in CH_2Cl_2 (212 mL), and DMAP (0.461 g, 3.8 mmol) was added followed by EDCI (9.529 g, 49.7 mmol). Within a minute, (cyanomethylene)triphenylphosphorane (**2**)

(15.000 g, 49.8 mmol) was added at once. The temperature rose to 30–35°C for about 10 min. Stirring was continued at rt for 4 h. Water (40 mL) was added, and the organic layer was washed consecutively with water (40 mL), sodium hydrogen carbonate (aq) (sat., 40 mL), and water (40 mL), and dried with magnesium sulfate (1 h). Filtration and concentration yielded a yellow–brown oil (26 g) which was purified by flash chromatography (1000 g, EtOAc/hex=50:50, 140 mL fractions, 38–49) to yield the desired product (**8**) as a solid off-white foam (17.539 g, 33.69 mmol, 88%): mp 50–60°C; TLC (EtOAc/hex=50:50): R_f 0.30; $[\alpha]_D^{20}=+26.18$ (*c* 1.23, CHCl$_3$); IR: 3390, 2990, 2160, 1705, 1575, 1495, 1430, 1100, 685 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 7.80–7.45 (m, 15, Ph$_3$P), 7.33 (m, 5, Ph), 5.57 (d, *J*=7.14 Hz, 1, NH), 5.10 (s (br), 2, CH$_2$O), 4.92 (m, 1, α-H), 2.08 (m, 1, β-H), 1.82 (m, 1, β-H), 0.93 (t, *J*=7.28 Hz, 3, CH$_3$); ^{13}C NMR (CDCl$_3$): δ 193.8 (d, J_{C-P}=2.47 Hz), 155.5, 136.5, 133.2 (d, J_{C-P}=10.49 Hz, 6), 133.0 (d, J_{C-P}=2.33 Hz, 3), 128.9 (d, J_{C-P}=12.60 Hz, 6), 128.1 (2), 127.5 (2), 127.5, 122.4 (d, J_{C-P}=93.90 Hz, 3), 120.7 (d, J_{C-P}=15.15 Hz), 66.0, 57.1 (d, J_{C-P}=9.08 Hz), 47.2 (d, J_{C-P}=126.55 Hz), 26.3, 9.1; MS: *m/z* 521 (100, M+H), 328 (90, Ph$_3$PC(CN)(CO)); HRMS: *m/z* calcd for $C_{32}H_{30}N_2O_3P$ (M+H)$^+$ 521.1994, found 521.1985.

3.2.2. (1-{1-[3-Cyano-1-ethyl-2-oxo-3-(triphenyl-λ⁵-phosphanylidene)-propylcarbamoyl]-2-methylpropyl-carbamoyl]-2-methylpropylcarbamoyl}-2-methylpro-pyl)-carbamic acid benzyl ester (11). Sodium carbonate (4.866 g, 46 mmol) was dissolved in water (28 mL), and H-Val-Val-OH (4.964 g, 22.95 mmol) was added. The resulting clear solution was cooled in an ice–water bath. A solution of benzyl chloroformate (3.6 mL, 4.3 g, 25 mmol) in dioxane (3.6 mL) was added drop-wise. After 18 h stirring at rt, the reaction mixture was extracted with CH_2Cl_2 (2×80 mL). The clear aqueous phase was acidified by the portion-wise addition of sodium hydrogen sulfate (11.4 g, 95.0 mmol). The precipitated product was extracted with EtOAc (4×100 mL). The combined extracts were washed with water (2×40 mL) and dried (MgSO$_4$, 1 h). Filtration and concentration afforded a white solid (6.862 g, 19.58 mmol, 85%). The product (**10**) was recrystallized from EtOAc/hex=50:50 (20 mL/g): mp 134.5–137.0°C; TLC (EtOAc/hex/AcOH=40:59:1): R_f 0.30; ^{1}H NMR (CDCl$_3$): δ 12.6 (s (br), 1, OH), 7.34 (m, 5, Ph), 6.64 (d, *J*=8.58 Hz, 1, NH), 5.56 (d, *J*=8.53 Hz, 1, NH), 5.11 (s, 2, CH$_2$O), 4.56 (dd, *J*=8.28 Hz, 4.80, 1, α-H), 4.07 (t, *J*=7.87 Hz, 1, α-H), 2.23 (m, 1, β-H), 2.08 (m, 1, β-H), 1.03–0.88 (m, 12, 4×CH$_3$). Cyanophosphorane **8** (17.233 g, 33.10 mmol) was dissolved in EtOAc (400 mL), and Pd/C (10% Pd, 34 g, 200% (w/w)) was added. The reaction mixture was stirred vigorously for 5 h at rt under an atmosphere of H$_2$, followed by filtration through Celite filter agent. Concentration furnished a pale-yellow crude product as a 1:1:2 mixture of unconverted starting material, a cyclized product and (**9**), which was used without further purification. A sample (1.5 g) was chromatographed (250 g, CH$_2$Cl$_2$/MeOH/Et$_3$N=95.3:3.7:1, 35 mL fractions, 23–27) to yield the pure product: TLC (CH$_2$Cl$_2$/MeOH/Et$_3$N=95.3:3.7:1): R_f 0.30; $[\alpha]_D^{20}=+14.55$ (*c* 1.01, CHCl$_3$); IR: 2990, 2950, 2155, 1575, 1475, 1425, 1210, 1100, 670 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 7.80–7.47 (m, 15, Ph$_3$P), 4.00 (dd, *J*=7.17, 5.33 Hz, 1, α-H), 1.89 (m, 1, β-H),

1.61 (m, 1, β-H), 1.45 (s (br), 2, NH$_2$), 1.00 (t, J=7.42 Hz, 3, CH$_3$); ^{13}C NMR (CDCl$_3$): δ 199.1, 133.4 (d, J_{C-P}=10.49 Hz, 6), 133.1 (d, J_{C-P}=2.54 Hz, 3), 129.1 (d, J_{C-P}=12.65 Hz, 6), 123.0 (d, J_{C-P}=94.27 Hz, 3), 121.8 (d, J_{C-P}=16.12 Hz), 57.8 (d, J_{C-P}=7.37 Hz), 46.9 (d, J_{C-P}=125.04 Hz), 28.8, 10.1; MS: m/z 387 (100, M+H), 328 (20, Ph$_3$PC(CN)(CO)); HRMS: m/z calcd for C$_{24}$H$_{24}$N$_2$OP (M+H)$^+$ 387.1626, found 387.1625. Fractions 30–38 from the above flash chromatography were concentrated to yield a white solid foam of (2S)-2-ethyl-5-imino-4-(triphenylphosphoranylidene)pyrrolidin-3-one, **9a**:[18] mp 70–80°C; TLC (CH$_2$Cl$_2$/MeOH/Et$_3$N=95.3:3.7:1): R_f 0.10; [α]$_D^{20}$=−5.54 (c 1.12, CHCl$_3$); IR: 3470, 2950, 1655, 1620, 1560, 1505, 1430, 1100, 670 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 9.16 (s (br), 1, NH), 8.99 (s (br), 1, NH), 7.84–7.62 (m, 15, Ph$_3$P), 3.96 (dd, J=6.28, 5.01 Hz, 1, α-H), 1.94 (m, 1, β-H), 1.77 (m, 1, β-H), 1.05 (t, J=7.41 Hz, 3, CH$_3$); ^{13}C NMR (CDCl$_3$): δ 197.0 (d, J_{C-P}=5.32 Hz), 169.3 (d, J_{C-P}=17.85 Hz), 134.4 (d, J_{C-P}=3.07 Hz, 3), 133.5 (d, J_{C-P}=10.86 Hz, 6), 129.9 (d, J_{C-P}=12.80 Hz, 6), 120.0 (d, J_{C-P}=92.05 Hz, 3), 63.7 (d, J_{C-P}=124.09 Hz), 63.5 (d, J_{C-P}=9.06 Hz), 24.5, 9.1; MS: m/z 387 (100, M+H); HRMS: m/z calcd for C$_{24}$H$_{24}$N$_2$OP (M+H)$^+$ 387.1626, found 387.1627. Cbz-Val-Val-OH (**10**) (6.456 g, 18.42 mmol) was dissolved in EtOAc (100 mL), and HOBt (4.980 g, 36.85 mmol) was added. To the resulting suspension, triethylamine (5.1 mL, 3.7 g, 36.6 mmol) was added, yielding a clear solution. The amino cyanophosphorane **9** (50%, 15.74 g, 20.4 mmol) was added as a solution in EtOAc (10 mL) and CH$_2$Cl$_2$ (2 mL). The mixture was cooled in an ice–water bath and EDCI (4.945 g, 25.80 mmol) was added. The stirring was continued at 0°C for 90 min, followed by 40 min at rt. Water (50 mL) was added. The organic layer was washed consecutively with sodium hydrogen carbonate (aq) (sat., 50 mL) and water (50 mL). The organic phase was dried (MgSO$_4$, 1 h) and filtered. Concentration yielded a semi-solid material. Purification by flash chromatography (75 g, EtOAc/hex=75:25, 25 mL fractions, 8–33) furnished a white solid which was recrystallized from EtOAc (375 mL) to yield the pure product (**11**) (9.122 g, 12.69 mmol, 69%): mp 202–203°C; TLC (EtOAc/hex=75:25): R_f 0.40; [α]$_D^{20}$=−18.12 (c 1.08, CHCl$_3$); IR: 3390, 2990, 2950, 2170, 1705, 1650, 1575, 1490, 1425, 1210, 685 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 7.76–7.47 (m, 15, Ph$_3$P), 7.32 (m, 5, Ph), 6.58 (d, J=7.19 Hz, 1, NH-Val), 6.55 (d, J=8.54 Hz, 1, NH-Abu), 5.51 (d, J=8.28 Hz, 1, NH-Val), 5.13 (q (br), ⟨J⟩=7.5 Hz, 1, α-H-Abu), 5.09 (d (AB), J=12.50 Hz, 1, CH$_2$O), 5.06 (d (AB), J=12.50 Hz, 1, CH$_2$O), 4.25 (t (br), ⟨J⟩=7.6 Hz, 1, α-H-Val), 4.03 (t (br), ⟨J⟩=7.4 Hz, 1, α-H-Val), 2.05 (m, 3, 2×β-H-Val, β-H-Abu), 1.77 (m, 1, β-H-Abu), 0.98–0.81 (m, 15, 5×CH$_3$); ^{13}C NMR (CDCl$_3$): δ 193.7 (d, J_{C-P}=2.52 Hz), 171.1, 170.0, 156.3, 136.4, 133.4 (d, J_{C-P}=10.70 Hz, 6), 133.2 (d, J_{C-P}=2.39 Hz, 3), 129.1 (d, J_{C-P}=12.64 Hz, 6), 128.3 (2), 127.8 (2), 127.8, 122.6 (d, J_{C-P}=94.25 Hz, 3), 120.7 (d, J_{C-P}=14.67 Hz), 66.6, 60.3, 58.3, 55.8 (d, J_{C-P}=9.00 Hz), 47.7 (d, J_{C-P}=125.68 Hz), 31.3, 31.2, 26.2, 19.0, 18.9, 18.2, 17.8, 9.4; MS: m/z 719 (100, M+H), 328 (100, Ph$_3$PC(CN)(CO)); HRMS: m/z calcd for C$_{42}$H$_{48}$N$_4$O$_5$P (M+H)$^+$ 719.3362, found 719.3356.

3.2.3. 2-(2-Amino-4-methyl-pentanoylamino)-3-methyl-butyric acid benzyl ester (12).

Boc-D-Leu-OH (2.774 g,

11.99 mmol), H-Val-OBn·TsOH (5.000 g, 13.18 mmol) and HOBt (1.620 g, 11.99 mmol) were suspended in CH$_2$Cl$_2$ (60 mL) and cooled in an ice–water bath. Triethylamine (2.00 mL, 1.45 g, 14.3 mmol) was added followed by EDCI (3.221 g, 16.8 mmol). Stirring was continued for 2 h at 0°C, followed by 4 h at rt. Citric acid (aq) (1%, 100 mL) was added, and the organic phase was washed consecutively with water (100 mL), sodium hydrogen carbonate (aq) (sat., 100 mL) and water (50 mL). The CH$_2$Cl$_2$ phase was dried (MgSO$_4$, 1 h), filtered, and concentrated to afford a clear colorless oil (5.088 g) which was purified by flash chromatography (250 g, EtOAc/hex=15:85, 70 mL fractions, 12–26). The resulting colorless oil (4.703 g, 11.18 mmol, 93%) solidified on standing: mp 81.5–83.0°C; TLC (EtOAc/hex=15:85): R_f 0.25; ^{1}H NMR (CDCl$_3$): δ 7.36 (m, 5, Ph), 6.69 (s (br), 1, NH), 5.20 (d (AB), J=12.25 Hz, 1, CH$_2$O), 5.12 (d (AB), J=12.25 Hz, 1, CH$_2$O), 4.80 (s (br), 1, NH), 4.57 (dd, J=8.70, 4.58 Hz, 1, α-H), 4.15 (s (br), 1, α-H), 2.20 (m, 1, CH-Val), 1.69 (m, 3, CH$_2$CH-D-Leu), 1.44 (s, 9, Bu^t), 0.93 (m, 6, 2×CH$_3$-Val), 0.92 (d, J=6.92 Hz, 3, CH$_3$-D-Leu), 0.85 (d, J=6.92 Hz, 3, CH$_3$-D-Leu). Boc-D-Leu-Val-OBn (4.329 g, 10.29 mmol) was dissolved in trifluoroacetic acid (47 mL) and stirred at rt for 45 min. Concentration furnished a colorless oil which was stripped with benzene (3×50 mL) to yield [(1R)-1-[(((1S)-1-[(benzyloxy)carbonyl]-2-methylpropyl)amino)-carbonyl]-3-methylbutyl]ammonium trifluoroacetate as a white solid (4.641 g, 10.68 mmol, >100%): mp 95–96°C; TLC (EtOAc/hex=75:25): R_f 0.10–0.20; [α]$_D^{20}$=−25.74 (c 1.01, CHCl$_3$); IR: 2950, 1775, 1725, 1665, 1535, 1170 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 8.00 (s (br), 3, NH$_3$), 7.63 (d, J=8.47 Hz, 1, NH-Val), 7.33 (m, 5, Ph), 5.18 (d (AB), J=12.12 Hz, 1, CH$_2$O), 5.06 (d (AB), J=12.12 Hz, 1, CH$_2$O), 4.53 (dd, J=8.56, 4.98 Hz, 1, α-H-Val), 4.30 (t (br), ⟨J⟩=7.1 Hz, 1, α-H-D-Leu), 2.19 (m, 1, CH-Val), 1.72 (m, 3, CH$_2$CH-D-Leu), 0.97 (d, J=5.89 Hz, 3, CH$_3$-Val), 0.93 (d, J=5.75 Hz, 3, CH$_3$-Val), 0.88 (d, J=6.86 Hz, 3, CH$_3$-D-Leu), 0.83 (d, J=6.88 Hz, 3, CH$_3$-D-Leu); ^{13}C NMR (CDCl$_3$): δ 171.7, 170.0, 161.3 (d, J_{C-F}=39.35 Hz), 134.8, 128.6 (2), 128.4 (2), 128.3, 115.4 (d, J_{C-F}=288.95 Hz), 67.6, 57.9, 52.5, 40.7, 30.8, 24.5, 22.1, 21.7, 18.9, 17.4; MS: m/z 321 (100, M+H); HRMS: m/z calcd for C$_{18}$H$_{29}$N$_2$O$_3$ (M+H)$^+$ 321.2178, found 321.2177. A solution of H-D-Leu-Val-OBn·TFA (4.585 g, 10.55 mmol) in CH$_2$Cl$_2$ (250 mL) was treated with sodium hydrogen carbonate (aq) (sat., 2×100 mL) followed by washing with water (2×100 mL). The organic phase was dried (MgSO$_4$, 1 h), filtered and concentrated to yield a colorless oil (**12**) (2.883 g, 9.00 mmol, 85%): TLC (EtOAc/hex=75:25): R_f 0.15; [α]$_D^{20}$=+5.12 (c 1.25, CHCl$_3$); IR: 3340, 2945, 1730, 1650, 1505, 1185, 1145 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 7.73 (d, J=8.97 Hz, 1, NH-Val), 7.35 (m, 5, Ph), 5.21 (d (AB), J=12.26 Hz, 1, CH$_2$O), 5.12 (d (AB), J=12.26 Hz, 1, CH$_2$O), 4.56 (dd, J=9.13, 4.81 Hz, 1, α-H-Val), 3.42 (dd (br), ⟨J⟩=10.0, 3.2 Hz, 1, α-H-D-Leu), 2.23 (m, 1, CH-Val), 1.71 (m, 2, CHCH-D-Leu), 1.44 (s (br), 2, NH$_2$), 1.35 (m, 1, CH-D-Leu), 0.95 (d, J=6.30 Hz, 3, CH$_3$), 0.93 (d, J=6.86 Hz, 3, CH$_3$), 0.92 (d, J=6.15 Hz, 3, CH$_3$), 0.88 (d, J=6.89 Hz, 3, CH$_3$-D-Leu); ^{13}C NMR (CDCl$_3$): δ 175.5, 171.7, 135.3, 128.4 (2), 128.2 (2), 128.1, 66.7, 56.6, 53.3, 43.7, 30.9, 24.6, 23.2, 21.1, 19.0, 17.5; MS: m/z 321 (100, M+H); HRMS: m/z calcd for C$_{18}$H$_{29}$N$_2$O$_3$ (M+H)$^+$ 321.2178, found 321.2180.

3.2.4. 2-(2-{3-[2-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-3-methyl-butyrylamino]-2-oxo-penta-noylamino}-4-methylpentanoylamino)-3-methylbutyric acid benzyl ester (13). The cyanophosphorane **11** (3.942 g, 5.48 mmol) was ozonized in CH_2Cl_2 (110 mL) at $-78°C$ for 34 min. The blue–green reaction mixture was purged with O_2 and N_2 for 8 and 10 min, respectively. To the resulting yellow mixture was added drop-wise a solution of H-D-Leu-Val-OBn (**12**) (2.636 g, 8.23 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred for 1 h before the cooling was discontinued. Concentration afforded a yellow oil which was subjected to flash chromatography (250 g+35 g for pre-adsorption, EtOAc/hex=40:60, 70 mL fractions, 16–25) to yield an off-white solid (1.330 g, 1.74 mmol, 32%). An aliquot (0.4 g) was re-chromatographed (20 g, CH_2Cl_2/MeOH=96:4, 4.5 mL fractions, 9–17) yielding **13** as a white solid (0.3 g) (<5% of the Pos epimer): mp 196–203°C; TLC (EtOAc/hex=50:50): R_f 0.40; $[\alpha]_D^{20}$=+12.00 (*c* 1.03, CHCl_3); ^1H NMR (CDCl_3): δ 7.47 (d, *J*=8.48 Hz, 1, NH), 7.34 (m, 10, 2×Ph), 7.07–6.57 (m, 3, 3×NH), 5.62 (d, *J*=6.02 Hz, 1, NH), 5.30 (m, 1, NH), 5.16 (d (AB), *J*=12.25 Hz, 1, CH_2O), 5.09 (m, 2, CH_2O), 5.08 (d (AB), *J*=12.25 Hz, 1, CH_2O), 4.64–4.51 (m, 2, α-H), 4.32 (m, 1, α-H), 4.08 (m, 1, α-H), 2.30–1.50 (m, 8, 7×β-H, γ-H-D-Leu), 1.08–0.77 (m, 27, 9×CH_3); ^{13}C NMR (CDCl_3): δ 196.1, 171.9, 171.8, 171.6, 171.2, 159.3, 156.5, 136.5, 135.2, 128.5, 128.4 (2), 128.3 (3), 127.9 (2), 127.8 (2), 67.0, 66.8, 60.5, 58.5, 57.3, 55.2, 52.0, 41.0, 31.2, 31.1, 31.0, 24.7, 24.7, 22.8, 22.1, 19.1, 19.0, 18.3, 18.2, 17.9, 17.6, 9.6; MS: *m/z* 766 (100, M+H); HRMS: *m/z* calcd for $C_{41}H_{60}N_5O_9$ (M+H)$^+$ 766.4391, found 766.4394.

3.2.5. 2-(2-{3-[2-(2-Amino-3-methylbutyrylamino)-3-methylbutyryl)amino]-2-oxo-pentanoylamino}-4-methylpentanoylamino]-3-methylbutyric acid (6). To a solution of **13** (0.275 g, 0.36 mmol) in CH_2Cl_2/MeOH=95:5 (25 mL) was added 10% Pd/C (0.14 g). The mixture was purged with N_2, before it was stirred vigorously for 24 h at rt under an atmosphere of H_2. The reaction mixture was filtered through Celite filter agent, which was rinsed with additional MeOH. Concentration yielded an off-white solid (0.167 g). Flash chromatography (5 g (C-18 SiO_2), water/MeOH=40:60, 1 mL fractions, 7–18) yielded synthetic poststatin (**6**) (15–20% of the Pos epimer) as an off-white solid (0.142 g, 0.26 mmol, 73%): mp 161–165°C; TLC (C-18 SiO_2) (water/MeOH=40:60): R_f 0.25; $[\alpha]_D^{20}$=+13.41 (*c* 0.88, AcOH); IR: 3210, 2950, 1660, 1535 cm^{-1}; ^1H NMR (major isomer) (DMSO-d_6): δ 8.55 (d, *J*=8.73 Hz, 1, NH), 8.38 (d, *J*=6.66 Hz, 1, NH), 8.12 (d, *J*=8.80 Hz, 1, NH), 7.97 (d, *J*=8.66 Hz, 1, NH), 4.95 (m, 1, α-H), 4.42 (dt, *J*=9.34 Hz, 4.77, 1, α-H), 4.28 (t (br), *J*=7.45 Hz, 1, α-H), 4.05 (dd, *J*=8.35, 5.44 Hz, 1, α-H), 3.54 (s (br), 3, NH_3), 3.22 (d, *J*=4.56 Hz, 1, α-H), 2.08–1.92 (m, 3, 3×β-H-Val), 1.75 (m, 1, β-H-Abu), 1.62 (m, 1, γ-H-D-Leu), 1.58–1.43 (m, 3, β-H-D-Leu, γ-H-D-Leu, β-H-Abu), 1.00–0.71 (m, 27, 9×CH_3); ^{13}C NMR (major isomer) (DMSO-d_6): δ 196.2, 173.0, 171.0, 171.0, 171.0, 160.5, 58.2, 57.5, 57.1, 54.8, 51.5, 40.8, 31.0, 30.8, 30.2, 24.3, 23.0, 22.7, 21.4, 19.2, 19.2, 19.2, 18.0, 17.9, 17.2, 10.4; MS: *m/z* 542 (100, M+H); HRMS: *m/z* calcd for $C_{26}H_{48}N_5O_7$ (M+H)$^+$ 542.3554, found 542.3549.

3.3. Experimental procedures used in the synthesis of eurystatin A

3.3.1. {3-Cyano-3-[diphenyl-(1-propenyl-buta-1,3-di-enyl-λ^5-phosphanylidene]-1-methyl-2-oxopropyl}-car-bamic acid benzyl ester (16). Cbz-ala-OH (**15**) (10.8120 g, 48.43 mmol) was dissolved in CH_2Cl_2 (270 mL) and DMAP (0.5916 g, 4.84 mmol) was added followed by EDCI (12.0710 g, 62.96 mmol). Within a minute, (cyanomethyl-ene)triphenylphosphorane (**2**) (15.3244 g, 50.86 mmol) was added at once. The temperature rose to 30–35°C for about 10 min. Stirring was continued at rt for 4 h. Water (15 mL) was added, and the organic layer was washed consecutively with water (15 mL), sodium hydrogen carbonate (aq) (sat., 15 mL), water (15 mL), and dried (MgSO_4, 1 h). Filtration and concentration yielded a yellow–brown oil (30.9150 g) which was purified by flash chromatography (1300 g, EtOAc/hex=50:50, 70 mL fractions, 58–90) to yield the desired product (**16**) as a white solid foam (20.8615 g, 41.18 mmol, 85%): mp 71–75°C; TLC (EtOAc/hex=50:50): R_f 0.33; $[\alpha]_D^{20}$=+18.96 (*c* 1.00, CHCl_3); IR: 3390, 3000, 2170, 1710, 1585, 1495, 1435, 1105 cm^{-1}; ^1H NMR (CDCl_3): δ 7.82–7.45 (m, 15, Ph_3P), 7.33 (m, 5, Ph), 5.67 (d, *J*=6.14 Hz, 1, NH), 5.11 (d (AB), *J*=13.65 Hz, 1, CH_2O), 5.08 (d (AB), *J*=13.65 Hz, 1, CH_2O), 4.94 (quint, $\langle J \rangle$=6.7 Hz, 1, α-H), 1.52 (d, *J*=6.68 Hz, 3, CH_3); ^{13}C NMR (CDCl_3): δ 194.6 (d, J_{C-P}=3.59 Hz), 155.3, 136.8, 133.4 (d, J_{C-P}=10.73 Hz, 6), 133.2 (3), 129.1 (d, J_{C-P}=13.09 Hz, 6), 128.3 (2), 127.7 (3), 122.5 (d, J_{C-P}=93.63 Hz, 3), 120.7 (d, J_{C-P}=14.91 Hz), 66.2, 52.4 (d, J_{C-P}=8.99 Hz), 46.6 (d, J_{C-P}=125.91 Hz), 19.6; MS: *m/z* 507 (85, M+H), 328 (100, Ph_3PC(CN)(CO)); HRMS: *m/z* calcd for $C_{31}H_{28}N_2O_3P$ (M+H)$^+$ 507.1837, found 507.1834.

3.3.2. 2-(3-Benzyloxycarbonylamino-2-oxobutyryl-amino)-4-methylpentanoic acid *tert*-butyl ester (19). The cyanophosphorane **16** (15.1871 g, 29.98 mmol) was ozonized in CH_2Cl_2 (600 mL) at $-78°C$ for 36 min (8 min at 2.02 mmol scale). The blue–green reaction mixture was purged with O_2 and N_2 for 4 and 8 min, respectively. To the resulting yellow solution of α,β-diketo nitrile **17** was added a solution of H-Leu-OBu^t (**18**) (5.3343 g, 28.48 mmol) in CH_2Cl_2 (36 mL). The mixture was stirred for 1 h before the cooling was discontinued. Concentration afforded a deep yellow oil which was stirred at rt for 24 h with a solution of silver nitrate in THF/water=4:1 (1 M, 300 mL, 300 mmol). Water (300 mL) was added to the dark greenish slurry, and the THF layer was washed with water (2×50 mL). The aqueous phases were extracted with CH_2Cl_2 (3×100 mL). The combined organic phases were dried (MgSO_4, 1 h), filtered, and concentrated to afford a clear yellow oil which was subjected immediately to flash chromatography (750 g, EtOAc/hex=25:75, 100 mL fractions, 17–50) to yield the desired product (**19**) (<5% of the Ala epimer) as a yellow oil (7.9877 g, 19.00 mmol, 67%): TLC (EtOAc/hex=25:75): R_f 0.30; $[\alpha]_D^{20}$=+11.49 (*c* 0.89, CHCl_3); IR: 3400, 2950, 1715, 1685, 1500, 1365, 1220, 1145 cm^{-1}; ^1H NMR (CDCl_3): δ 7.36 (m, 5, Ph), 7.25 (d, *J*=8–9 Hz, 1, NH), 5.44 (d, *J*=7.37 Hz, 1, NH), 5.20 (quint, $\langle J \rangle$=7.4 Hz, 1, α-H-Ala), 5.13 (d (AB), *J*=11.57 Hz, 1, CH_2O), 5.09 (d (AB), *J*=11.57 Hz, 1, CH_2O), 4.48 (dd, *J*=8.37 Hz, 5.49, 1, α-H-Leu), 1.72–1.53 (m, 3, CH_2CH-Leu), 1.48 (s, 9, Bu^t), 1.47 (d, *J*=7.4 Hz, 3, CH_3-Ala), 0.96 (d, *J*=6.27 Hz, 3,

CH$_3$-Leu), 0.96 (d, J=6.17 Hz, 3, CH$_3$-Leu); ^{13}C NMR (CDCl$_3$): δ 196.1, 170.7, 158.3, 155.4, 136.2, 128.4 (2), 128.1 (2), 128.0, 82.4, 66.9, 51.9, 51.4, 41.6, 27.9 (3), 24.9, 22.6, 22.0, 17.9; MS: m/z 421 (30, M+H), 321 (100, M+H–CH$_2$CMe$_2$–CO$_2$); HRMS: m/z calcd for C$_{22}$H$_{33}$N$_2$O$_6$ (M+H)$^+$ 421.2338, found 421.2339.

3.3.3. 2-[3-(2-Benzyloxycarbonylamino-5-*tert*-butoxy-carbonylaminopentanoylamino)-2-oxo-butyrylamino]-4-methylpentanoic acid *tert*-butyl ester (22).

A solution of α-keto amide **19** (0.1953 g, 0.46 mmol) in CH$_2$Cl$_2$ (10 mL) was stirred vigorously with 10% Pd/C (98 mg) under an atmosphere of H$_2$. The stirring was continued for 40 min at rt. A sample was filtered through Celite filter agent. Concentration furnished the crude (**20**) as a yellow oil: TLC (EtOAc/hex=75:25): R_f 0.05; $[\alpha]_D^{20}$=+4.17 (c 1.32, CH$_2$Cl$_2$); IR: 3370, 2950, 1718, 1665, 1510, 1365, 1240, 1145 cm^{-1}; ^{1}H NMR (CDCl$_3$): complex; ^{13}C NMR (CDCl$_3$): complex; MS: m/z 289 (100, M+3H); HRMS: m/z calcd for C$_{14}$H$_{29}$N$_2$O$_4$ (M+3H)$^+$ 289.2127, found 289.2125 (1 ppm). The above reaction mixture was cooled in an ice–water bath and briefly purged with N$_2$. HOBt (0.1255 g, 0.93 mmol), Cbz-Orn(Boc)-OH (**21**) (0.1872 g, 0.51 mmol), and EDCI (0.1247 g, 0.65 mmol) were added as solids in the order listed, and stirring was continued for 2.5 h at rt. The reaction mixture was filtered through Celite before citric acid (aq) (1%, 10 mL) was added. The organic layer was washed with water (2×10 mL), dried (MgSO$_4$, 0.5 h), filtered, and concentrated to furnish a pale yellow oil. The crude product was dissolved in CH$_2$Cl$_2$ (30 mL) and concentrated with SiO$_2$ (3 g). Flash chromatography (20 g, EtOAc/hex=50:50, 5 mL fractions, 15–30) yielded the desired product (**22**) as a white solid foam (0.1791 g, 0.28 mmol, 61%): mp 58–62°C; TLC (EtOAc/hex=50:50): R_f 0.40; $[\alpha]_D^{20}$=−1.65 (c 0.97, CHCl$_3$); IR: 3420, 2980, 1740, 1720, 1690, 1515, 1380, 1235, 1165 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 7.33 (m, 5, Ph), 7.30 (d, J=8–9 Hz, 1, NH-Leu), 7.01 (s (br), 1, NH-Ala), 5.64 (d, J=8.12 Hz, 1, NH-Orn), 5.27 (quint, $\langle J \rangle$=7.1 Hz, 1, α-H-Ala), 5.09 (s (br), 2, CH$_2$O), 4.85 (s (br), 1, NH-δ-Orn), 4.45 (dt, J=5.48 Hz, 8.43, 1, α-H-Leu), 4.37 (s (br), 1, α-H-Orn), 3.27 (m, 1, CH$_2$N), 3.08 (m, 1, CH$_2$N), 1.86 (m, 1, β-H-Orn), 1.71–1.51 (m, 6, CH$_2$CH-Leu, CH$_2$CH-Orn), 1.46 (s, 9, Boc), 1.43 (d, J=6–7 Hz, 3, CH$_3$-Ala), 1.42 (s, 9, Bu^t), 0.95 (d, J=6.10 Hz, 3, CH$_3$-Leu), 0.94 (d, J=6.06 Hz, 3, CH$_3$-Leu); ^{13}C NMR (CDCl$_3$): δ 195.7, 171.5, 170.9, 158.7, 156.4, 156.3, 136.2, 128.4 (2), 128.0, 127.9 (2), 82.3, 79.0, 66.8, 53.5, 51.3, 50.3, 41.4, 39.4, 30.2, 28.3 (3), 27.9 (3), 26.0, 24.9, 22.6, 22.0, 16.6; MS: m/z 635 (25, M+H), 435 (70, M+H–CH$_2$CMe$_2$–CO$_2$), 479 (100, M+H−2×CH$_2$CMe$_2$–CO$_2$); HRMS: m/z calcd for C$_{32}$H$_{51}$N$_4$O$_9$ (M+H)$^+$ 635.3656, found 635.3674. The reaction was repeated on a 7.07 g (16.8 mmol) scale yielding 43% of the purified tripeptide **22**.

3.3.4. [3-Cyano-1-methyl-2-oxo-3-(triphenyl-λ^5-phosphanylidene)-propyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester (24).

Fmoc-ala-OH (**23**) (4.7013 g, 15.10 mmol) was suspended in CH$_2$Cl$_2$ (84 mL) and DMAP (0.1845 g, 1.51 mmol) was added followed by EDCI (3.7633 g, 19.63 mmol). (Cyanomethylene)triphenyl-phosphorane (**2**) (4.7777 g, 15.86 mmol) was added at once. The clear yellow reaction mixture was stirred at rt for 4 h. Water (15 mL) was added, and the organic layer was washed consecutively with water (15 mL), sodium hydrogen carbonate (aq) (sat., 15 mL), water (15 mL), and (MgSO$_4$, 1 h). Filtration and concentration yielded a yellow–brown oil which was purified by flash chromatography (300 g, EtOAc/hex=50:50, 70 mL fractions, 26–62) to yield the desired product (**24**) as a white solid foam (4.8394 g, 8.14 mmol, 54%): mp 100–105°C; TLC (EtOAc/hex=50:50): R_f 0.30; $[\alpha]_D^{20}$=+19.82 (c 1.13, CHCl$_3$); IR: 3390, 3005, 2170, 1715, 1585, 1495, 1435, 1105 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 7.76 (d, J=7.50 Hz, 2, Ar-Fmoc), 7.72–7.45 (m, 17, Ph$_3$P, Ar-Fmoc), 7.39 (t, J=7.42 Hz, 2, Ar-Fmoc), 7.29 (t, J=7.43 Hz, 2, Ar-Fmoc), 5.78 (d, J=7.04 Hz, 1, NH), 4.98 (quint, $\langle J \rangle$=6.9 Hz, 1, α-H), 4.35 (quint, $\langle J \rangle$=6.7 Hz, 2, CH$_2$O), 4.21 (t, $\langle J \rangle$=7.2 Hz, 1, CH-Fmoc), 1.57 (d, J=6.76 Hz, 3, CH$_3$); ^{13}C NMR (CDCl$_3$): δ 194.6, 155.3, 144.1, 143.9, 141.2 (2), 133.4 (d, J_{C-P}=10.45 Hz, 6), 133.3 (d, J_{C-P}=2 Hz, 3), 129.2 (d, J_{C-P}=12.72 Hz, 6), 127.5 (2), 126.9 (2), 125.2 (2), 122.5 (d, J_{C-P}=93.76 Hz, 3), 120.7 (d, J_{C-P}=15.21 Hz), 119.8 (2), 66.5, 52.4 (d, J_{C-P}=9.21 Hz), 47.1, 46.7 (d, J_{C-P}=125.56 Hz), 19.7; MS: m/z 595 (65, M+H), 328 (100, Ph$_3$PC(CN)(CO)); HRMS: m/z calcd for C$_{38}$H$_{32}$N$_2$O$_3$P (M+H)$^+$ 595.2150, found 595.2146.

3.3.5. [4-*tert*-Butoxycarbonylamino-1-(1-{3-cyano-1-methyl-3-[(1-methylene-hexa-2,4-dienyl)-(1-methyl-hexa-1,3,5-trienyl)-phenyl-λ^5-phosphanylidene]-2-oxo-propylamino}-ethyl)-butyl]-carbamic acid benzyl ester (26).

Cyanophosphorane **24** (2.0828 g, 3.50 mmol) was stirred in piperidine (35 mL, 30.14 g, 354 mmol) for 15 min at rt. Concentration afforded a white solid. The crude material was concentrated twice from CH$_2$Cl$_2$ (35 mL) and triethylamine (2 mL) to afford the piperidine-free 4-amino-2-[(1-methylene-hexa-2,4-dienyl)-(1-methyl-hexa-1,3,5-trienyl)-phenyl-λ^5-phosphanylidene]-3-oxo-pentanenitrile (**25**): TLC (EtOAc/hex=75:25): R_f 0.05; IR: 2950, 2870, 2110, 1555, 1410, 1080 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 7.87–7.40 (m, 15, Ph$_3$P), 4.19 (q, J=6.85 Hz, 1, α-H), 2.06 (s (br), 2, NH$_2$), 1.41 (d, J=6.87 Hz, 3, CH$_3$); ^{13}C NMR (CDCl$_3$): δ 199.7, 133.5 (d, J_{C-P}=9.48 Hz, 6), 133.2 (d, J_{C-P}=2 Hz, 3), 129.2 (d, J_{C-P}=12.59 Hz, 6), 123.1 (d, J_{C-P}=93.84 Hz, 3), 121.8 (d, J_{C-P}=16.08 Hz), 52.5 (d, J_{C-P}=8.18 Hz), 45.9 (d, J_{C-P}=125.66 Hz), 22.0; MS: m/z 373 (70, M+H), 328 (30, Ph$_3$PC(CN)(CO)); HRMS: m/z calcd for C$_{23}$H$_{22}$N$_2$OP (M+H)$^+$ 373.1470, found 373.1481 (3 ppm). Amine **25** was dissolved in CH$_2$Cl$_2$ (35 mL) along with HOBt (0.9466 g, 7.01 mmol). Cbz-Orn(Boc)-OH (**21**) (1.4117 g, 3.85 mmol) and EDCI (0.9400 g, 4.90 mmol) were added. The stirring was continued for 20 h at rt. The reaction mixture was washed consecutively with citric acid (aq) (1%, 15 mL), water (15 mL), sodium hydrogen carbonate (aq) (sat., 15 mL), and water (15 mL). The combined organic phases were dried (MgSO$_4$, 1 h), filtered, and concentrated to yield a red–brown oil. The product (**26**) was isolated by flash chromatography (60 g, EtOAc/hex=75:25, 25 mL fractions, 15–31) as an off-white solid foam (1.4045 g, 1.95 mmol, 56%): mp 91–97°C; TLC (EtOAc/hex=75:25): R_f 0.25; $[\alpha]_D^{20}$=+2.27 (c 1.06, CHCl$_3$); IR: 3400, 2995, 2165, 1700, 1665, 1580, 1495, 1430, 1220 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 7.75–7.45 (m, 15, Ph$_3$P), 7.34 (m, 5, Ph), 6.62 (d, J=6.68 Hz, 1, NH-Ala), 5.49 (d, J=7.58 Hz, 1, NH-Orn), 5.08 (m, 3, α-H-Ala, CH$_2$O), 4.59 (s (br), 1, δ-NH-Orn), 4.17 (s (br), 1, α-H-Orn), 3.09 (m, 1, CH$_2$N), 3.00 (m,

1, CH$_2$N), 1.81 (m, 1, β-H-Orn), 1.61 (m, 1, β-H-Orn), 1.53 (d, J=6.85 Hz, 3, CH$_3$), 1.45 (m, 2, γ-CH$_2$-Orn), 1.41 (s, 9, Boc); ^{13}C NMR (CDCl$_3$): δ 194.3 (d, J_{C-P}=2 Hz), 170.2, 155.9, 155.8, 136.3, 133.4 (d, J_{C-P}=9.93 Hz, 6), 133.3 (d, J_{C-P}=2.3 Hz, 3), 129.2 (d, J_{C-P}=12.69 Hz, 6), 128.4 (2), 127.9 (2), 127.9, 122.5 (d, J_{C-P}=93.51 Hz, 3), 120.6 (d, J_{C-P}=15.53 Hz), 78.8, 66.7, 54.2, 51.2 (d, J_{C-P}=9.05 Hz), 46.8 (d, J_{C-P}=126.89 Hz), 39.8, 30.4, 28.3 (3), 25.4, 18.9; MS: m/z 721 (45, M+H), 328 (100, Ph$_3$PC(CN)(CO)); HRMS: m/z calcd for C$_{41}$H$_{46}$N$_4$O$_6$P (M+H)$^+$ 721.3155, found 721.3156.

3.3.6. 2-[3-(2-Benzyloxycarbonylamino-5-*tert*-butoxy-carbonylaminopentanoylamino)-2-oxobutyrylamino]-4-methylpentanoic acid *tert*-butyl ester (22).

Cyanophosphorane **26** (0.5562 g, 0.77 mmol) was ozonized in CH$_2$Cl$_2$ (15 mL) at −78°C until the reaction mixture turned blue–green (16 min). After purging with O$_2$ and N$_2$, the yellow solution of α,β-diketo nitrile **27** was quenched with a solution of H-Leu-OBu^t (**18**) (0.1373 g, 0.73 mmol) in CH$_2$Cl$_2$ (1 mL). The cooling was discontinued after 1 h. Concentration furnished a yellow oil which was stirred at rt for 24 h with a solution of silver nitrate in THF/water=4:1 (1 M, 7.7 mL, 7.7 mmol). Water (10 mL) was added, and the THF layer was washed with water (2×2 mL). The aqueous phases were extracted with CH$_2$Cl$_2$ (3×5 mL). The combined organic phases were dried (MgSO$_4$, 1 h), filtered, and concentrated to afford a yellow oil which was immediately flash chromatographed (30 g, EtOAc/hex=50:50, 10 mL fractions, 5–14) to yield the desired product (**22**) (<5% of the Ala epimer) as a yellow oil (0.3557 g, 0.56 mmol, 76%).

3.3.7. (3-Isobutyl-7-methyl-2,5,6,9-tetraoxo-1,4-8-triaza-cyclotridec-10-yl)-carbamic acid benzyl ester (29).

Tripeptide **22** (3.4554 g, 5.44 mmol) was dissolved in CH$_2$Cl$_2$ (136 mL) and TFA (136 mL) was added at rt. The solution was stirred for 90 min and concentrated. The pale yellow oil was dissolved and concentrated repeatedly from CH$_2$Cl$_2$ (3×136 mL) to yield (**28**) as a white solid foam (3.3225 g, 5.61 mmol, 103% (including solvent traces)): mp 50–57°C; TLC (EtOAc/hex=75:25): R_f 0.00; [α]$_D^{20}$=−28.98 (c 0.92, CHCl$_3$); IR: 2950, 1780, 1720, 1670, 1525, 1225, 1175 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 11.91 (s (br), 1, OH), 8.81 (d, J=8.34 Hz, 1, NH-Leu), 8.35 (d, J=6.48 Hz, 1, NH-Ala), 7.75 (s (br), 3, NH$_3$), 7.44 (d, J=8.34 Hz, 1, NH-Orn), 7.36 (m, 5, Ph), 5.00 (m, 3, α-H-Ala, CH$_2$O), 4.27 (dt, J=3.77 Hz, 8.35, 1, α-H-Leu), 4.08 (m, 1, α-H-Orn), 2.77 (m, 2, CH$_2$N), 1.69 (m, 2, CH$_2$-Leu), 1.67–1.48 (m, 5, γ-CH-Leu, β-CH$_2$-Orn, γ-CH$_2$-Orn), 1.25 (d, J=7.32 Hz, 3, CH$_3$-Ala), 0.86 (d, J=5.98 Hz, 3, CH$_3$-Leu), 0.83 (d, J=5.84 Hz, 3, CH$_3$-Leu); ^{13}C NMR (DMSO-d_6): δ 196.8, 173.2, 171.7, 160.9, 158.8 (q, J_{C-F}=36.64 Hz), 156.1, 137.1, 128.5 (2), 128.0, 127.9 (2), 115.8 (q, J_{C-F}=290.87 Hz), 65.7, 53.7, 50.4, 49.8, 39.3, 38.7, 29.1, 24.6, 23.8, 22.9, 21.2, 15.5; MS: m/z 479 (100, M+H−TFA); HRMS: m/z calcd for C$_{23}$H$_{35}$N$_4$O$_7$ (M+H−TFA)$^+$ 479.2506, found 479.2501. The acid **28** was dissolved in degassed DMF (1087 mL) and cooled to −15°C (ethylene glycol/CO$_2$ (s)). Diphenylphosphoryl azide (5.86 mL, 7.48 g, 27.2 mmol) and sodium hydrogen carbonate (4.57 g, 54.4 mmol) were added, and the suspension was stirred briskly for 40 h. Filtration and concentration yielded

an off-white semisolid material which was subjected to flash chromatography (500 g, CH$_2$Cl$_2$/MeOH/AcOH=95:4:1, 175 mL fractions, 21–45) to give a white solid **29** (2.2192 g, 4.82 mmol, 89%). The acetic acid remaining in the purified product was removed by filtration through a second column (110 g, CH$_2$Cl$_2$/MeCN=50:50) which was packed using the eluent with 1% acetic acid added. The column was purged thoroughly with CH$_2$Cl$_2$/MeCN=50:50 before the product was loaded as a supersaturated solution in MeCN/water=75:25. Recrystallization was accomplished from MeCN/water=75:25: mp 245–248°C; TLC (CH$_2$Cl$_2$/MeOH=95:5): R_f 0.25; [α]$_D^{20}$=−145.12 (c 0.25, DMSO); IR (DMSO): 3475, 1725, 1695, 1685, 1675, 1665, 1545, 1250 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 8.80 (d, J=7.79 Hz, 1, NH-Leu), 8.21 (d, J=8.12 Hz, 1, NH-Ala), 7.40 (d, J=6.98 Hz, 1, α-NH-Orn), 7.35 (m, 6, Ph, δ-NH-Orn), 5.03 (d, J=12.62 Hz, 1, CH$_2$O), 4.98 (d, J=12.62 Hz, 1, CH$_2$O), 4.68 (quint, $\langle J \rangle$=7.12 Hz, 1, α-H-Ala), 4.10 (td (br), $\langle J \rangle$=8.6, 5.4 Hz, 1, α-H-Leu), 3.86 (q (br), $\langle J \rangle$=5.5 Hz, 1, α-H-Orn), 3.04 (m, 2, CH$_2$N), 1.64–1.42 (m, 7, CH$_2$CH-Leu, CH$_2$CH$_2$-Orn), 1.15 (d, J=6.76 Hz, 3, CH$_3$-Ala), 0.88 (d, J=6.18 Hz, 3, CH$_3$-Leu), 0.82 (d, J=6.27 Hz, 3, CH$_3$-Leu); ^{13}C NMR (DMSO-d_6): δ 197.9, 172.4, 171.4, 165.0, 155.9, 136.9, 128.3 (2), 127.8, 127.7 (2), 65.5, 53.3, 52.2, 49.6, 38.6, 36.5, 28.0, 24.5, 24.3, 22.7, 21.4, 15.1; MS: m/z 461 (80, M+H), 185 (100); HRMS: m/z calcd for C$_{23}$H$_{33}$N$_4$O$_6$ (M+H)$^+$ 461.2400, found 461.2399. Concentration from methanol provided the corresponding methyl hemiketal as a single diastereomer; HRMS: m/z calcd for C$_{24}$H$_{37}$N$_4$O$_7$ (M+H+CH$_3$OH)$^+$ 493.2662, found 493.2655.

3.3.8. (*E*)-6-Methyl-2-heptenoic acid (31).

To a vigorously stirred suspension of pyridinium chlorochromate (39.5906 g, 183.66 mmol) in CH$_2$Cl$_2$ (270 mL) was added a solution of 4-methylpentanol (**20**) (12.5112 g, 122.44 mmol) in CH$_2$Cl$_2$ (30 mL) over a period of 45 min. The temperature rose to 35°C, and the reaction mixture turned dark brown–orange. After a total reaction time of 6 h, ether (300 mL) was added. The resulting greenish solution was passed through Florisil (100 g), and the granulate from the ether precipitation was washed with additional ether (3×30 mL) which was likewise filtered. The resulting green–yellow solution of 4-methylpentanal was concentrated to a volume of 350 mL and added drop-wise over a 30 min period to a solution of (*tert*-butoxycarbonylmethylene)triphenylphosphorane in CH$_2$Cl$_2$ (350 mL) at rt. After an additional 2 h stirring, the reaction mixture was concentrated to a volume of 230 mL and used without further purification. In a separate experiment the preparation was repeated on the same scale, and the crude product ((*E*)/(*Z*)=95:5) was purified by fractional distillation to yield *tert*-butyl (*E*)-6-methyl-2-heptenoate (19.5365 g, 98.52 mmol, 80% (57% of the pure (*E*)-isomer in the late distillate) as a colorless oil: bp 70°C, 0.5 mmHg; TLC (EtOAc/hex=5:95): R_f 0.50; IR: 2940, 1695, 1640, 1460, 1385, 1370, 1310, 1245, 1155 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 6.84 (dt, J=15.58 Hz, 6.94, 1, β-H), 5.72 (dt, J=15.58 Hz, 1.50, 1, α-H), 2.15 (q, $\langle J \rangle$=8.4 Hz, 2, γ-H), 1.56 (nonet, $\langle J \rangle$=6.7 Hz, 1, ε-H), 1.46 (s, 9, Bu^t), 1.31 (q, $\langle J \rangle$=6.9 Hz, 2, δ-H), 0.88 (d, J=6.62 Hz, 6, 2×CH$_3$); ^{13}C NMR (CDCl$_3$): δ 166.1, 148.2, 122.8, 79.8, 37.1, 29.9, 28.1 (3), 27.5, 22.3 (2); MS: m/z 199 (10, M+H); EA: calcd for C$_{12}$H$_{22}$O$_2$: C, 72.68; H, 11.18, found: C, 72.96; H, 11.05. The red–pink solution

of crude **23** from above was mixed with TFA (230 mL) and stirred for 2 h at rt. Concentration furnished a green oil that was subjected to fractional distillation. The product was collected as a colorless oil (15.3868 g, 108.2 mmol, 88%, 72% of the pure (*E*)-isomer in the late distillate): mp ≈10°C; bp 94°C, 0.5 mmHg; TLC (EtOAc/hex=15:85): R_f 0.15; IR: 2950, 1685, 1640, 1410, 1375, 1360, 1280 cm^{-1}; ^{1}H NMR (CDCl$_3$): δ 11.61 (s (br), 1, OH), 7.10 (dt, *J*=15.59 Hz, 6.96, 1, β-H), 5.84 (dt, *J*=15.59 Hz, 1.56, 1, α-H), 2.25 (dq, ⟨*J*⟩=7.0, 1.6 Hz, 2, γ-H), 1.59 (nonet, ⟨*J*⟩=6.7 Hz, 1, ε-H), 1.37 (q, ⟨*J*⟩=7.2 Hz, 2, δ-H), 0.91 (d, *J*=6.55 Hz, 6, 2×CH$_3$); ^{13}C NMR (CDCl$_3$): δ 172.4, 152.7, 120.5, 36.9, 30.2, 27.5, 22.3 (2); MS: *m/z* 143 (100, M+H), 125 (60, M+H−H$_2$O).

3.3.9. *N*-[(3*S*,7*S*,10*S*,*E*)-7-Methyl-3-(2-methylpropyl)-2,5,6,9-tetraoxo-1,4,8-triazacyclotridec-10-yl]-6-methyl-2-heptenamide (14a).

A solution of **29** (1.0489 g, 2.28 mmol) in CH$_2$Cl$_2$/MeOH=90:10 (50 mL) was stirred vigorously with 10% Pd/C (0.2621 g) for 15 h at rt under an atmosphere of H$_2$. A sample was filtered through Celite filter agent. Concentration yielded a white solid consisting of the methyl hemiketal of 10-amino-3-isobutyl-7-methyl-1,4,8-triaza-cyclotridecane-2,5,6,9-tetraone (**30**) as a single diastereomer: mp 226–229°C; TLC (CH$_2$Cl$_2$/MeOH=95:5): R_f 0.00; $[\alpha]_D^{20}$=−85.19 (*c* 0.22, CH$_2$Cl$_2$/MeOH=90:10); IR (DMSO): 3400, 1715, 1685, 1665, 1650, 1535, 1240 cm^{-1}; ^{1}H NMR (CD$_2$Cl$_2$/CD$_3$OD): δ 4.37 (q, *J*=6.69 Hz, 1, α-H-Ala), 4.26 (s (br), 5, NH/H$_2$O), 4.01 (q (br), ⟨*J*⟩=2.8 Hz, 1, α-H-Leu), 3.88 (s (br), 1, α-H-Orn), 3.11 (m, 1, CH$_2$N), 3.08 (m, 1, CH$_2$N), 1.95 (m, 1, CH$_2$-Leu), 1.75–1.50 (m, 6, Me$_2$CHCH-Leu, CH$_2$CH$_2$-Orn), 1.13 (d, *J*=6.84 Hz, 3, CH$_3$-Ala), 0.95 (d, *J*=6.12 Hz, 3, CH$_3$-Leu), 0.87 (d, *J*=6.05 Hz, 3, CH$_3$-Leu); ^{13}C NMR (CD$_2$Cl$_2$/CD$_3$OD): δ 173.3, 171.3, 168.7, 99.1, 53.2, 52.3, 50.7, 49.0 (sept, J_{C-D}=21.48), 38.5, 37.0, 28.2, 25.2, 24.4, 23.0, 21.7, 13.4 (NMR data were obtained directly from the reaction mixture of an experiment conducted in CD$_2$Cl$_2$/CD$_3$OD. The NMR spectra were in addition recorded in DMSO-d_6 displaying a 60:40 mixture of the free ketone and its methyl hemiketal); MS: *m/z* 359 (100, M+H+CH$_3$OH), 327 (15, M+H); HRMS: *m/z* calcd for C$_{15}$H$_{27}$N$_4$O$_4$ (M+H)$^+$ 327.2032, found 327.2031, *m/z* calcd for C$_{16}$H$_{31}$N$_4$O$_5$ (M+H+CH$_3$OH)$^+$ 359.2294, found 359.2287. The colorless reaction mixture was purged with N$_2$. EDCI (0.6549 g, 3.42 mmol) and (*E*)-6-methyl-2-heptenoic acid (**31**) (0.4048 g, 2.85 mmol) were added, and stirring was continued for 3 h at rt. The reaction mixture was filtered through Celite and concentrated. The crude product was subjected to flash chromatography (200 g, CH$_2$Cl$_2$/MeOH/AcOH=95:4:1, 70 mL fractions, 14–41) to give eurystatin A (**14a**) as a white solid (0.5727 g, 1.27 mmol, 56%). The acetic acid remaining in the purified product was removed by filtration through a second column (30 g, CH$_2$Cl$_2$/MeCN=50:50), which was packed using the eluent with 1% acetic acid added. The column was purged thoroughly with CH$_2$Cl$_2$/MeCN=50:50 before the product was loaded as a supersaturated solution in MeCN/water=75:25. Recrystallization was accomplished from MeCN/water=75:25: mp 291–294°C; TLC (CH$_2$Cl$_2$/MeOH=95:5): R_f 0.20; $[\alpha]_D^{20}$=−132.54 (*c* 0.25, DMSO); IR (DMSO): 3460, 3290, 1725, 1675, 1645, 1545 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 8.80 (d, *J*=7.77 Hz, 1, NH-Leu), 8.19

(d, *J*=8.45 Hz, 1, NH-Ala), 8.00 (d, *J*=7.46 Hz, 1, α-NH-Orn), 7.41 (t (br), ⟨*J*⟩=6.4 Hz, 1, δ-NH-Orn), 6.60 (dt, *J*=15.44, 6.98 Hz, 1, CH=CC=O), 6.02 (dt, *J*=15.44, 1.45 Hz, 1, C=CHC=O), 4.72 (quint, ⟨*J*⟩=7.5 Hz, 1, α-H-Ala), 4.19 (q (br), ⟨*J*⟩=6.1 Hz, 1, α-H-Orn), 4.10 (td (br), ⟨*J*⟩=8.5, 5.7 Hz, 1, α-H-Leu), 3.09 (m, 1, CH$_2$N), 3.03 (m, 1, CH$_2$N), 2.12 (q, ⟨*J*⟩=7.0 Hz, 2, C=CCH$_2$), 1.66–1.42 (m, 8, CH$_2$CH-Leu, CH$_2$CH$_2$-Orn, CHMe$_2$), 1.27 (q, ⟨*J*⟩=7.4 Hz, 2, C=CCCH$_2$), 1.14 (d, *J*=6.81 Hz, 3, CH$_3$-Ala), 0.89 (d, *J*=6.25 Hz, 3, CH$_3$-Leu), 0.86 (d, *J*=6.62 Hz, 6, 2×CH$_3$), 0.82 (d, *J*=6.34 Hz, 3, CH$_3$-Leu); ^{13}C NMR (DMSO-d_6): δ 197.9, 172.2, 171.5, 165.2, 165.0, 143.0, 124.0, 52.3, 51.1, 49.5, 38.7, 36.9, 36.5, 29.1, 28.3, 26.9, 24.5, 24.2, 22.7, 22.3, 21.4, 15.1; MS: *m/z* 469 (50, M+H+H$_2$O), 451 (100, M+H); HRMS: *m/z* calcd for C$_{23}$H$_{39}$N$_4$O$_5$ (M+H)$^+$ 451.2920, found 451.2922. Concentration from methanol provided the corresponding methyl hemiketal as a single diastereomer; HRMS: *m/z* calcd for C$_{24}$H$_{43}$N$_4$O$_6$ (M+H+CH$_3$OH)$^+$ 483.3182, found 483.3179.

3.4. Experimental procedures used in the synthesis of phebestin

3.4.1. 6-*tert*-Butoxycarbonylamino-2-isopropyl-4,5-dioxo-7-phenylheptanoic acid benzyl ester (34).

A solution of 2.4 g (4.4 mmol) cyanophosphorane **33** in 100 mL CH$_2$Cl$_2$ at −78°C was ozonized for 15 min until the solution turned deep yellow/green. Then the solution was purged with N$_2$ for 15 min until light yellow, and to this reaction was added 1.09 g (5.26 mmol) H-Val-OBn in 10 mL CH$_2$Cl$_2$. The mixture was stirred for 1 h at −78°C and then slowly warmed to rt. The solvent was removed by rotary evaporation and the crude product was purified by silica gel chromatography (EtOAc/hex=1:1) to give 1.3 g (62%) as a white solid (**34**): mp 110–112°C; $[\alpha]_D^{20}$=−39.05 (*c* 1.4, CHCl$_3$); IR (CHCl$_3$) 3417, 3325, 3250, 1702 and 1501 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 0.92 (d, *J*=6.9 Hz, 3H), 0.96 (d, *J*=6.9 Hz, 3H), 1.41 (s, 9H), 2.30 (m, 1H), 3.04 (dd, *J*=13.7, 6.6 Hz, 1H), 3.30 (dd, *J*=13.8, 4.7 Hz, 1H), 4.58 (dd, *J*=4.6, 4.3 Hz, 1H), 5.10 (m, 1H), 5.19 (d, *J*=12.2 Hz, 1H), 5.26 (d, *J*=12.2 Hz, 1H), 5.36 (m, 1H), 7.12 (m, 2H), 7.25–7.41 (m, 8H); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 17.7, 19.1, 28.3, 31.4, 37.6, 56.7, 57.4, 67.4, 80.1, 127.1, 128.5, 128.66, 128.74, 129.5, 135.2, 135.8, 136.2, 155.0, 159.1, 170.7 195.5; HRMS calcd for C$_{27}$H$_{35}$N$_2$O$_6$ (M+H)$^+$ 483.2495, found 483.2491.

3.4.2. 6-*tert*-Butoxycarbonylamino-5-hydroxy-2-iso-propyl-4-oxo-7-phenylheptanoic acid benzyl ester (35).

Zinc borohydride in diethyl ether (4.34 mL, 0.64 mmol) was added to a solution of compound **34** (0.150 g, 0.321 mmol) in THF at −78°C and the resulting mixture was stirred for 1 h. The reaction mixture was then quenched with water, neutralized with dilute acetic acid and extracted with CH$_2$Cl$_2$. The organic extracts were washed with water and brine, dried (MgSO$_4$), and concentrated in vacuo. The resultant residue (92:8 mixture of diastereomers) was purified by PLC (EtOAc/hex=1:1) affording **35** (0.131 g, 82%): mp 119.5–121°C; $[\alpha]_D^{20}$=+10.2 (*c* 1.0, CHCl$_3$); IR (film) ν_{max} 2442, 3406, 3274, 1737,1681 and 1514 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 0.84 (d, *J*=6.9 Hz, 3H), 0.90 (d, *J*=6.8 Hz, 3H), 1.39 (s, 9H), 2.20 (m, 1H), 3.05 (dd, *J*=13.6, 6.5 Hz, 1H), 3.20 (m, 1H), 3.96 (m, 1H), 4.18 (s

(br), 1H), 4.60 (dd, *J*=9.2, 4.8 Hz, 1H), 4.98 (m, 1H), 5.15 (d, *J*=12.1 Hz, 1H), 5.21 (d, *J*=12.1 Hz, 1H), 5.79 (s (br), 1H), 7.22–7.29 (m, 10H); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 17.5, 19.2, 28.3, 31.4, 36.1, 56.0, 56.8, 67.1, 74.6, 80.6, 126.7, 128.4, 128.5, 128.6, 129.3, 135.4, 138.2, 157.7, 171.5, 172.7; HRMS calcd for C$_{27}$H$_{37}$N$_2$O$_6$ (M+H)$^+$ 485.2651, found 485.2657. Anal. calcd for C$_{27}$H$_{36}$N$_2$O$_6$: C, 66.92; H, 7.49; N, 5.78. Found: C, 66.84; H, 7.36; N, 5.74.

3.4.3. 2-(6-*tert*-Butoxycarbonylamino-5-hydroxy-2-isopropyl-4-oxo-7-phenylheptanoylamino)-3-phenylpropionic acid benzyl ester (36).

A solution of **35** (0.100 g, 0.201 mmol) in anhydrous CH$_3$OH (40 mL) was treated with 10% Pd/C (15 mg, 0.15 wt equiv.) and stirred at 20°C under H$_2$ (1 atm) for 2 h. The reaction mixture was filtered through a pad of Celite, washed with 10% CH$_3$OH/CH$_2$Cl$_2$ and concentrated in vacuo to afford the carboxylic acid as a crude solid, which was immediately used in the next step without further purification. A solution of the crude acid, H-Phe-OBn (0.227 mmol) and 1-hydroxybenzotriazole (30.7 mg, 0.23 mmol) in anhydrous CH$_2$Cl$_2$ (50 mL) at 0°C was treated with triethylamine (46.0 mg, 0.45 mmol) and the resultant mixture stirred to homogeneity. EDCI (44.0 mg, 0.227 mmol) was then added and the solution was allowed to warm to rt and stirred overnight. The reaction was quenched with sat. NH$_4$Cl, washed with H$_2$O, NaHCO$_3$, brine, dried (MgSO$_4$, 1 h), and the solvent removed. The crude solid was purified by silica gel chromatography (5% CH$_3$OH in CH$_2$Cl$_2$) to give **36** (0.105 g, 83% for 2 steps): mp 134–136°C; [α]$_D^{20}$=−6.18 (*c* 3.4, CHCl$_3$); IR (film) ν_{max} 3439, 3088, 2970, 1739, 1682, 1512 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 0.84 (d, *J*=6.7 Hz, 3H), 0.89 (d, *J*=6.7 Hz, 3H), 1.37 (s, 9H), 2.11 (m, 1H), 3.06 (m, 4H), 4.07 (m, 2H), 4.3 (m, 1H), 4.92 (dd, *J*=13.7, 6.3 Hz, 1H), 5.07 (d, *J*=12.5 Hz, 1H), 5.14 (d, *J*=12.5 Hz, 1H), 5.25 (m, 1H), 5.90 (s (br), 1H), 7.02 (m, 2H), 7.15–7.35 (m, 13H), 7.51 (d, *J*=8.7 Hz, 1H); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 18.0, 19.4, 28.3, 30.8, 36.7, 37.8, 53.4, 55.6, 58.1, 67.3, 73.8, 80.2, 126.6, 127.1, 128.2, 128.3, 128.5, 128.58, 128.64, 129.3, 129.5, 135.1, 135.7, 138.3, 157.2, 171.0, 171.2 172.9; HRMS calcd for C$_{36}$H$_{46}$N$_3$O$_7$ (M+H)$^+$ 632.3336, found 632.3336.

3.4.4. Phebestin, 2-[2-(3-amino-2-hydroxy-4-phenylbutyrylamino)-3-methylbutyrylamino]-3-phenylpropionic acid (37).

A solution of **36** (44 mg, 0.070 mmol) in anhydrous CH$_3$OH (5 mL) was treated with 5% Pd/C (7 mg, 0.15 wt equiv.) and stirred at 20°C under H$_2$ (1 atm) for 2 h. The reaction mixture was filtered through a pad of Celite, washed with 10% CH$_3$OH/CH$_2$Cl$_2$ and concentrated in vacuo to afford the carboxylic acid as a crude solid, which was immediately used in the next step without further purification. The crude solid was then treated at rt with 50% TFA in CH$_2$Cl$_2$ (2 mL) for 15 min and the solvent was removed immediately under reduced pressure to afford a white salt. The salt was treated with NH$_4$OH followed by decantation to yield phebestin (**37**) (24.6 mg, 80% for 2 steps) as a white solid: mp 188–191°C, (lit. mp 190–192°C); [α]$_D^{20}$=−11.9 (*c* 1.0, HOAc), (lit. [α]$_D^{27}$=−12.6 (*c* 0.54, HOAc)); IR (film) ν_{max} 3380, 3220, 2960, 1655 cm^{-1}; ^{1}H NMR (DMSO-*d*$_6$, 500 MHz) δ 0.78 (d, *J*=6.3 Hz, 3H), 0.81 (d, *J*=6.4 Hz, 3H), 1.99 (m, 1H), 2.67 (dd, *J*=13.4,

7.1 Hz, 1H), 2.84 (m, 2H), 3.03 (dd, *J*=13.9, 5.2 Hz, 1H), 3.26 (m, 1H), 3.86 (d, *J*=2.4 Hz, 1H), 4.13 (m, 1H), 4.33 (m, 1H), 7.10–7.32 (m, 10H), 7.79 (d, *J*=8.3 Hz, 1H), 8.21 (d, *J*=7.1 Hz, 1H); ^{13}C NMR (DMSO-*d*$_6$, 125 MHz) δ 17.8, 19.2, 30.7, 36.8, 38.9, 53.8, 54.6, 57.2, 69.9, 126.2, 126.4, 128.0, 128.4, 129.1, 129.3, 137.7, 137.8, 170.4, 171.4, 172.8; HRMS calcd for C$_{24}$H$_{32}$N$_3$O$_5$: (M+H)$^+$ 442.2342, found 442.2340.

3.5. Experimental procedures used in the synthesis of probestin

3.5.1. [1-Benzyl-3-cyano-2-oxo-3-(triphenyl-λ^5-phosphanylidene)-propyl]-carbamic acid *tert*-butyl ester (33).

To a solution of 0.4 g (3.3 mmol) DMAP and 6.4 g (33 mmol) EDCI in 120 mL dry CH$_2$Cl$_2$ was added 8.2 g (31 mmol) *N*-Boc-D-phenylalanine and 11.1 g (36.8 mmol) (cyanomethylene)triphenylphosphorane (**2**). The reaction was stirred for 5 h at rt and then quenched with 50 mL H$_2$O, washed with 50 mL sat. NaCl, and dried (MgSO$_4$, 1 h). The solvent was removed in vacuo and the crude solid purified by silica gel column chromatography (EtOAc/hex=1.5:1) to give 14.9 g (88%) of **33** as a white solid: mp 185–187°C; [α]$_D^{20}$=−32.8 (C 2.0, CHCl$_3$); IR (CHCl$_3$) 3435, 3025, 2190, 1715, 1590 cm^{-1}. ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.39 (s, 9H), 3.05 (m, 1H), 3.36 (m, 1H), 5.15 (m, 2H), 7.23 (m, 5H), 7.48–7.65 (m, 15H); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 28.4, 38.8, 57.1, 79.0, 121.2 (d, *J*=14.7 Hz), 122.7 (d, *J*=93.6 Hz), 126.5, 128.2, 129.3 (d, *J*=13.0 Hz), 129.9, 133.3, 133.7 (d, *J*=10.4 Hz), 137.2, 155.3, 193.7; HRMS calcd for C$_{34}$H$_{33}$N$_2$O$_3$P: (M+H)$^+$ 549.2307, found 549.2303.

3.5.2. 2-(3-*tert*-Butoxycarbonylamino-2-oxo-4-phenylbutyrylamino)-4-methylpentanoic acid benzyl ester (38).

A solution of 0.55 g (1.0 mmol) cyanophosphorane **33** in 20 mL CH$_2$Cl$_2$ at −78°C was ozonolyzed for 15 min until the solution turned deep yellow/green. The solution was purged with N$_2$ for 5 min until light yellow and then treated with H-Leu-OBn (0.22 g, 1.0 mmol) in 10 mL CH$_2$Cl$_2$. The mixture was stirred for 1 h at −78°C and then slowly warmed to rt. The solvent was removed, and the crude product was purified by silica gel chromatography (EtOAc/hex=1:1) to give **38** (0.29 g, 58%) as a white solid: mp 96–98°C; [α]$_D^{20}$=−49.7 (C 1.4, CHCl$_3$); IR (CHCl$_3$) 3420, 3020, 2985, 1715, 1505 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 0.94 (d, *J*=5.6 Hz, 6H), 1.41 (s, 9H), 1.65 (m, 3H), 3.08 (dd, *J*=13.0, 6.1 Hz, 1H), 3.30 (dd, *J*=13.0, 4.5 Hz,1H), 4.68 (m, 1H), 5.04 (m, 1H), 5.22 (s, 2H), 5.38 (m, 1H), 7.10 (m, 2H), 7.25 (m, 3H), 7.37 (m, 5H); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 21.8, 22.8, 24.9, 28.3, 37.6, 41.2, 51.0, 56.7, 67.4, 80.1, 127.1, 128.4, 128.6, 128.7, 129.5, 135.2, 135.7, 155.0, 158.9, 171.6, 195.4; HRMS calcd for C$_{28}$H$_{36}$N$_2$O$_6$: (M+K)$^+$ 535.2210, found 535.2203.

3.5.3. 2-(3-*tert*-Butoxycarbonylamino-2-hydroxy-4-phenylbutyrylamino-4-methylpentanoic acid benzyl ester (39).

Zinc borohydride in diethyl ether (2.7 mL, 0.4 mmol) was added to the solution **38** (0.1 g, 0.2 mmol) in THF at −78°C and the resulting mixture stirred for 1 h. The reaction mixture was quenched with water, neutralized with dilute acetic acid and extracted with CH$_2$Cl$_2$. The organic extracts were washed with water and brine, dried (MgSO$_4$),

and concentrated in vacuo. The residue which was a 93:7 mixture of diastereomers was purified by PLC (EtOAc/hex=1:1) affording 0.085 g (85%) of **39** as a white solid: mp 140–142°C; $[\alpha]_D^{20}$=+2.97 (*c* 2.2, CHCl$_3$); IR (CHCl$_3$) 3400, 3385, 3235, 2900, 1740, 1693, 1650, 1528 and 1500 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 0.9 (m, 6H), 1.40 (s, 9H), 1.64 (m, 3H), 3.03 (m, 1H), 3.17 (m, 1H), 3.98 (m, 1H), 4.17 (dd, *J*=6.8, 2.7 Hz, 1H), 4.67 (m, 1H), 5.00 (d, *J*=7.6 Hz, 1H), 5.17 (s, 2H), 5.67 (m, 1H) and 7.21–7.40 (m, 11H); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 21.7, 23.1, 24.8, 28.3, 36.7, 41.2, 50.4, 50.5, 55.5, 67.2, 73.4, 80.0, 126.5, 127.0, 127.5, 128.2, 128.5, 128.7, 129.4, 135.4, 138.3, 157.0, 172.8 and 172.9; HRMS calcd for C$_{28}$H$_{38}$N$_2$O$_6$: (M+H)$^+$ 499.2808, found 499.2806.

3.5.4. 1-{1-[2-(3-*tert*-Butoxycarbonylamino-2-hydroxy-4-phenylbutyrylamino)-4-methyl-pentanoyl]-pyrrolidine-2-carbonyl}-pyrrolidine-2-carboxylic acid benzyl ester (40).

A solution of **39** (0.33 g, 0.66 mmol) in anhydrous CH$_3$OH (40 mL) was treated with 10% Pd/C (50 mg, 0.15 wt equiv.) and stirred at 20°C under H$_2$ (1 atm) for 2 h. The reaction mixture was filtered through a pad of Celite, washed with 10% CH$_3$OH/CH$_2$Cl$_2$ and concentrated in vacuo to afford the carboxylic acid as a white solid (0.257 g), which was immediately used in the next step without further purification. A solution of the crude acid (0.257 g), H-Pro-Pro-Obn (0.235 g, 0.692 mmol) and 1-hydroxybenzotriazole (94 mg, 0.692 mmol) in anhydrous CH$_2$Cl$_2$ (50 mL) at 0°C was treated with triethylamine (140.1 mg, 1.28 mmol) and the resultant mixture stirred to homogeneity. EDCI (133 mg, 0.69 mmol) was then added and the solution was allowed to warm to rt and stirred overnight. The reaction was quenched with sat. NH$_4$Cl, washed with H$_2$O, NaHCO$_3$, brine, dried (MgSO$_4$, 1 h), and the solvent was removed. The crude solid was purified by silica gel chromatography (5% CH$_3$OH in CH$_2$Cl$_2$) to give **40** (0.391 g, 89% for two steps): mp 80–83°C; $[\alpha]_D^{20}$=−78.3 (*c* 1.2, CHCl$_3$); IR (CHCl$_3$) 3425, 3380, 3290, 2950, 1740, 1705, 1651, 1635 and 1495 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 0.95 (d, *J*=6.0 Hz, 3H), 0.97 (d, *J*=5.2 Hz, 3H), 1.37 (s, 9H), 1.55–1.76 (m, 3H), 1.77–2.03 (m, 6H), 2.03–2.21 (m, 3H), 2.95 (m, 2H), 3.55 (m, 1H), 3.70 (m, 2H), 3.80 (m, 1H), 4.05 (m, 1H), 4.11 (m, 1H), 4.55 (m, 1H), 4.65 (m, 1H), 4.83 (dd, *J*=14.0, 8.8 Hz, 1H), 5.00 (d, *J*=12.2 Hz, 1H), 5.06 (m, 1H), 5.22 (d, *J*=12.2 Hz, 1H), 5.92 (d, *J*=4.7 Hz, 1H), 7.14–7.43 (m, 10H) and 7.57 (d, *J*=8.8 Hz, 1H); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 21.5, 23.6, 24.6, 24.8, 28.0, 28.1, 28.3, 28.5, 37.0, 40.7, 46.5, 47.3, 48.7, 55.2, 58.1, 58.7, 66.9, 72.2, 126.3, 126.9, 128.2, 128.3, 128.4, 128.5, 128.6, 129.6, 135.6, 138.4, 155.9, 169.9, 171.6, 171.9, 172.3 and 174.9; HRMS calcd for C$_{38}$H$_{52}$N$_4$O$_8$: (M+H)$^+$ 503.2869, found 503.2872.

3.5.5. Probestin, 1-{1-[2-(3-amino-2-hydroxy-4-phenyl-butyrylamino)-4-methylpentanoyl]-pyrrolidine-2-carbonyl}-pyrrolidine-2-carboxylic acid (41).

A solution of **40** (28 mg, 0.041 mmol) in anhydrous CH$_3$OH (2 mL) was treated with 5% Pd/C (4 mg, 0.15 wt equiv.) and stirred at 20°C under H$_2$ (1 atm) for 2 h. The reaction mixture was filtered through a pad of Celite, washed with 10% CH$_3$OH/CH$_2$Cl$_2$ and concentrated in vacuo to afford the carboxylic acid as a crude solid, which was immediately used in the next step without further purification. The crude solid was

treated at rt with 50% TFA in CH$_2$Cl$_2$ (1 mL) for 15 min and the solvent removed immediately in vacuo affording a white salt. The salt was treated with NH$_4$OH followed by decantation to yield probestin (**41**) as a white solid (17 mg, 84% for 2 steps): mp 167–170°C, (lit. mp 168–170°C); $[\alpha]_D^{25}$=−117 (*c* 1.0, CH$_3$OH), (lit. $[\alpha]_D^{20}$=−112 (*c* 0.9, CH$_3$OH)); IR (CHCl$_3$) ν_{max} 3398, 2959, 1640 cm^{-1}; ^{1}H NMR (CD$_3$OD, 300 MHz) δ 0.98 (*d*=6.4 Hz, 6H), 1.61 (m, 2H), 1.75 (m, 1H), 2.01 (m, 6H), 2.25 (m, 2H), 2.91 (dd, *J*=13.8, 6.9 Hz, 1H), 3.10 (dd, *J*=13.8, 8.0 Hz, 1H), 3.30 (br, s, 1H), 3.63 (m, 2H), 3.75 (m, 2H), 3.86 (m, 1H), 4.10 (d, *J*=3.3 Hz, 1H), 4.40 (m, 1H), 4.63 (m, 2H) and 7.31 (m, 5H); ^{13}C NMR (CD$_3$OD, 75 MHz) δ 22.0, 23.6, 25.8, 25.9, 29.1, 30.0, 36.4, 40.8, 48.2, 51.3, 56.2, 59.7, 60.1, 69.7, 128.5, 130.0, 130.6, 136.8, 172.3, 172.7, 173.4, 175.2; HRMS calcd for C$_{26}$H$_{38}$N$_4$O$_6$ (M+H)$^+$ 503.2869, found 503.2872.

3.6. Experimental procedures used in the synthesis of bestatin

3.6.1. Bestatin, 2-(3-amino-2-hydroxy-4-phenylbutyryl-amino)-4-methylpentanoic acid (42).

Compound **39** (63 mg, 0.13 mmol) in a solution of 2 M HCl in EtOAc (15 mL) was stirred at rt for 2.5 h. The resulting precipitate was filtered and washed with EtOAc and then hexanes yielding a white salt. A solution of this salt in anhydrous CH$_3$OH (10 mL) was treated with 10% Pd/C (9 mg, 0.15 wt equiv.) and stirred at 20°C under H$_2$ (1 atm) for 2.5 h. The reaction mixture was filtered through a pad of Celite, washed with CH$_3$OH and concentrated in vacuo to afford a white solid which was crystallized from a mixture of 5% hexanes in EtOAc to yield bestatin hydrochloride (**42**)·HCl (40 mg, 92%): mp 210–214°C, (lit. mp 212–218°C); $[\alpha]_D^{20}$=−15.2 (*c* 0.83, 1 M HCl), (lit. $[\alpha]_D^{22}$=−15.5 (*c* 1.0, 1 M HCl)); IR (KBr) ν_{max} 3650–2250, 1723, 1660, 1604, 1532 and 1498 cm^{-1}; ^{1}H NMR (CD$_3$OD, 300 MHz) δ 0.93 (d, *J*=5.5 Hz, 6H), 1.69 (m, 3H), 2.91 (dd, *J*=13.5, 6.6 Hz, 1H), 3.10 (dd, *J*=13.5, 8.6 Hz, 1H), 3.74 (m, 1H), 4.04 (d, *J*=2.4 Hz, 1H), 4.18 (m, 1H) and 7.2–7.4 (m, 5H).
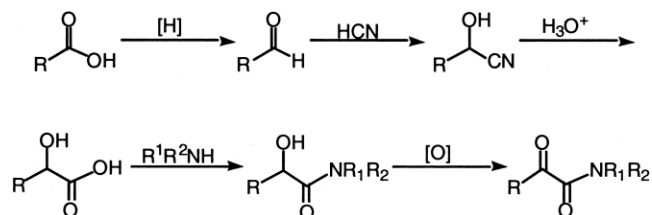
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16. The earlier general route to α-keto amides from carboxylic acids followed the sequence shown below


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18. A side product in the deprotection of **8** was shown to be the pyrrolidin-3-one **9a** formed most probably by the intra-molecular addition of the free amino group to the nitrile (see Section 3.1.2)

9a
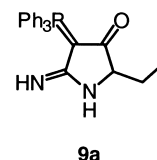
19. The synthetic poststatin was identical in all respects to a sample kindly provided by Dr Y. Muraoka.

20. (a) Toda, S.; Obi, Y.; Numata, K.-i.; Hamagishi, Y.; Tomita, K.; Komiyama, N.; Kotake, C.; Furumai, T.; Oki, T. *J. Antibiot.* **1992**, *45*, 1573–1579. (b) Toda, S.; Kotake, C.; Tsuno, T.; Narita, Y.; Yamasaki, T.; Konishi, M. *J. Antibiot.* **1992**, *45*, 1580–1586. (c) Suzuki, K.; Toda, S.; Furumai, T.; Fukagawa, Y.; Oki, T. *J. Antibiot.* **1994**, *47*, 982–991.

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22. (*E*)-6-Methyl-2-heptenoic acid (**31**) was prepared from 4-methyl-1-pentanol by oxidation to the corresponding aldehyde. Wittig olefination with (*tert*-butoxycarbonyl-methylene)triphenylphosphorane afforded *tert*-butyl-(*E*)-6-methyl-2-heptenoate as a 95:5 mixture of geometric isomers. Deprotection with TFA followed by fractional distillation gave the isomerically pure (*E*)-acid **31**
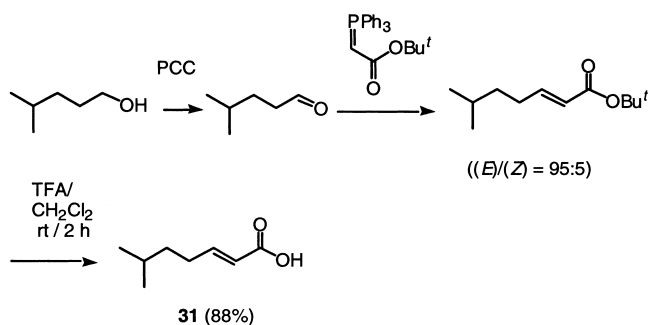
23. A noteworthy feature of this synthesis pertains to the preservation of the stereochemical integrity throughout, as shown by the appearance of a single set of signals in the ^{1}H and ^{13}C NMR spectra of all intermediates. Additional evidence was obtained from a 1:1 mixture of **22** and its alanine epimer prepared by equilibration with triethylamine. Cyclization, according to the protocol shown in Scheme 6, furnished **29** and its alanine epimer in a ratio of 1:1. This control experiment demonstrated unambiguously that the stereochemistry of **29** was intact.

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26. We thank Dr Takaaki, Institute of Microbial Chemistry, Tokyo for samples of phebestin and probestin.

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