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Synthesis and Biological Evaluation of Pretubulysin and Derivatives

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Dedicated to Dr. Dieter Häbich on the occasion of his 60th birthday

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Pretubulysin, a biosynthetic precursor of the tubulysins, shows potent biological activity in the subnanomolar range towards various tumor cell lines. Its activity is only slightly less than those of the structurally more complex tubulysins. With a straightforward synthesis to hand, pretubulysin is an ideal lead structure for the development of tubulysin-based anticancer drugs

Introduction

The tubulysins (**1**) are an interesting family of peptidic secondary metabolites (Figure 1) of myxobacteria, first isolated and characterized by Höfle and Reichenbach et al.[1] The high cytotoxicities of these compounds are the result of inhibition of tubulin polymerization, leading to apoptosis.[2] This mode of action differs from those of paclitaxel and the epothilones, and the tubulysins also exhibit significantly higher bioactivities, with IC_{50} values in the low nM range towards various cancer cell lines.[3]

The structure of the tubulysins can be subdivided into four amino acid building blocks, two of them represented by an N-terminal *N*-methylated D-pipecolic acid (D-Mep), connected to L-Ile, the only proteinogenic amino acid. The metabolites also contain two unusual amino acids: tubuvaline (Tuv), a thiazole amino acid derived from valine, and a C-terminal extended aromatic γ-amino acid. In some tubulysin family members this γ-amino acid is tubuphenylalanine (Tup, R^2 = H), whereas others incorporate tubutyrosine (Tut, R^2 = OH). All tubulysins contain an acetoxy function at the α-position of Tuv. The most unusual structural feature is the rare bis-acyl *N*,*O*-acetal moiety on the Tuv-amide, which is found in all highly potent derivatives. Tubulysins lacking this unique structure exhibit substantially reduced biological activity.[4] However, this unit poses the greatest challenge to synthetic chemists. It is thus not surprising that only one total synthesis of tubulysin D (**1D**), by the Ellman group, has been reported to date.[5] Their studies also indicated, however, that the *N*,*O*-acetal per se is not necessary for high biological activity, a result supported by work by Wipf et al.:[6] compounds incorporating a simple *N*-methylamide at the same position can be equally potent,[7] and only when the *N*-substituent is removed completely a significant drop in activity is observed.[4,8]

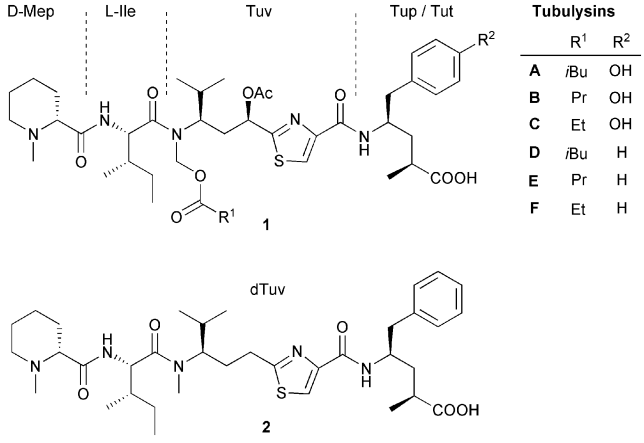

Figure 1. Selected tubulysins (**1**) and pretubulysin (**2**).

SAR studies have also focused on the N-terminal amino acid Mep (which can be replaced by *N*-methylsarcosine) and the C-terminal Tup, which can be substituted with a wide range of alternative functionalities with retention of biological activity.[7a]

During studies on the biosynthesis of the tubulysins, we identified a range of biosynthetic intermediates in extracts of the producing strain *Angiococcus disciformis* An d48, ap-

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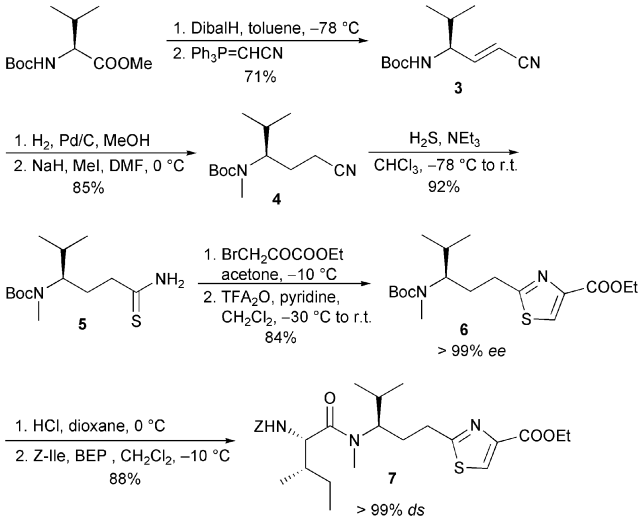
parently generated by alternative modes of operation of the polyketide synthase (PKS)/nonribosomal peptide synthetase (NRPS) multienzyme assembly line.[10] One of the characterized compounds was pretubulysin (**2**, Figure 1), a structure proposed earlier to be the first enzyme-free intermediate in the pathway. Pretubulysin lacks the acetoxy group (dTuv instead of Tuv) and the *N*,*O*-acetal functionality, which is consistent with these groups being introduced by post-assembly line enzymes. The SAR studies on the tubulysin analogues suggest that pretubulysin should also retain significant biological activity. In addition, because it lacks the configurationally labile acetoxy group, pretubulysin is an ideal lead structure for the development of tubulysin-based anticancer drugs. We therefore pursued the total synthesis of pretubulysin[9] and adapted our route to afford a range of derivatives.

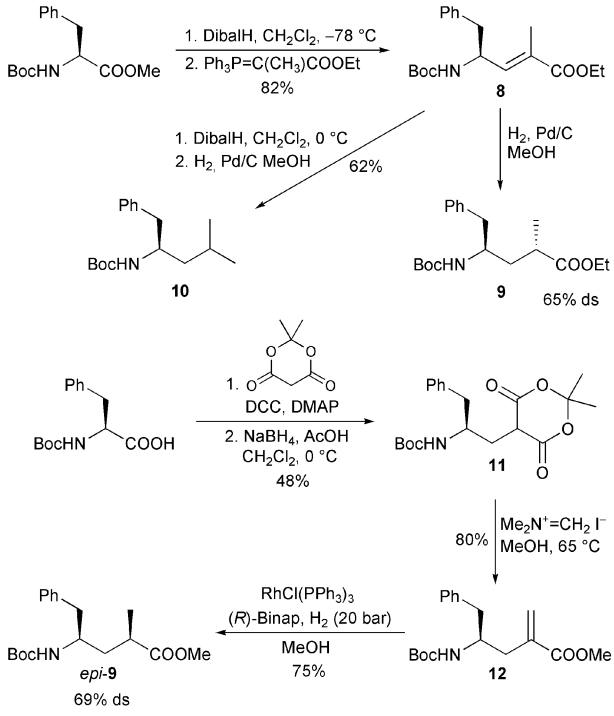
Results and Discussion

The synthesis of the central Ile-dTuv unit is shown in Scheme 1. From the starting *N*-Boc-protected valine ester, a DibalH reduction and in situ Wittig reaction of the resulting aldehyde gave rise to the enantiomerically pure unsaturated nitrile **3**. Catalytic hydrogenation and subsequent *N*-methylation to afford **4** proceeded without racemization. The nitrile functionality was then converted into the thioamide (compound **5**),[11] which was subjected to a Hantzsch thiazole synthesis. Trifluoroacetic acid anhydride (TFA_2O) was added to the hydroxythiazoline intermediate to form the thiazole ring (compound **6**).[12] Cleavage of the Boc protecting group and coupling with Z-Ile gave rise to the required dipeptide **7**. BEP (2-bromo-1-ethylpyridinium tetrafluoroborate) was used as a coupling reagent to avoid the racemization frequently observed in couplings of *N*-methyl amino acids and to achieve a high yield.[13]



Scheme 1. Synthesis of the Ile-dTuv fragment.

Despite the apparent simplicity of the Tup moiety, the stereoselective introduction of the α-methyl group is not a trivial issue and has represented a significant challenge in previous synthetic studies. Because our attempts to introduce the methyl group through an enolate alkylation of the ester modified with the Evans auxiliary[14] were unsuccessful (due to lactamization), we decided to use the more straightforward approach of catalytic hydrogenation. The required α,β-unsaturated ester **8** was easily obtained from protected phenylalanine by DibalH reduction/Wittig olefination, as reported for dTuv (Scheme 2). No epimerization was observed in this one-pot reaction, whereas isolation of the aldehyde intermediate resulted in nearly complete racemization. Catalytic hydrogenation gave rise to the Tup derivative **9** as a 2:1 diastereomeric mixture. Hydrogenation of the free acid, as performed by Wipf,[12b] unfortunately brought no improvement in selectivity. Interestingly, reduction of **8** to the corresponding allyl alcohol and catalytic hydrogenation resulted in a reductive deoxygenation and the formation of the protected amine **10**.[15]



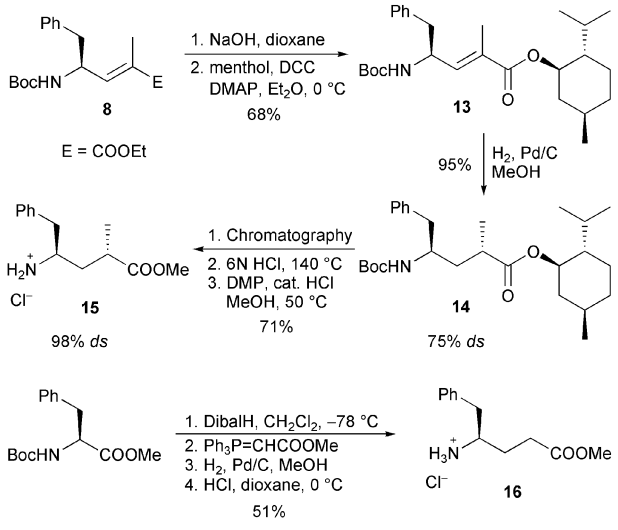
Scheme 2. Synthetic attempts directed towards Tup.

This prompted us also to investigate homogeneous hydrogenations. Unfortunately, no hydrogenation of **8** was observed in the presence of Wilkinson's catalyst, and so we focused on the reduction of substrate **12** with a methylene side chain. Compound **12** was obtained from Boc-Phe by coupling with Medrum's acid[16] and subsequent reduction of the keto group with $NaBH_4$.[17] Treatment of the substituted Medrum's acid **11** with Eschenmoser's salt gave rise

to the required α-substituted acrylate **12**. Unfortunately, attempts to obtain higher selectivity with this substrate were unsuccessful.

Although the hydrogenation with this sterically less hindered substrate proceeded cleanly, in the presence of (*R*)-Binap as chiral ligand the "wrong" diastereomer *epi*-**9** was formed preferentially. To make sure that this was not the result of a mismatch situation we also investigated the reaction in the presence of the enantiomeric ligand, but with (*S*)-Binap the situation was even worse. The hydrogenation was very slow and **9** was obtained as a more or less isomeric mixture (45% ds).

Because Zanda et al. had reported the separation of the corresponding menthyl esters of **9** by chromatography,[8a] we converted **8** into the menthyl ester **13** (Scheme 3). Its hydrogenation provided **14** with a slightly better selectivity, with respect to the required diastereomer, than in the case of **8**. After separation of the diastereomers, cleavage of the protecting groups yielded **15** in enantiomerically pure form.[8a] To evaluate whether the α-methyl group has any influence on the biological activity, we also synthesized the demethyl derivative (dTup) **16** in an analogous manner.



Scheme 3. Synthesis of Tup (**15**) and dTup (**16**).

This readily available analogue was also used to investigate the final steps of our synthesis, firstly with a simplified analogue containing *N*-methylsarcosine instead of D-Mep (Scheme 4). The dipeptide **7** was saponified at the ester functionality, and coupling with **16** gave rise to the tripeptide **17** without significant lactam formation. The Z protecting group could not be removed by catalytic hydrogenolysis (due to the thiazole moiety), so it was instead disconnected under acidic conditions. Coupling with *N*-methylsarcosine, activated as its pentafluorophenyl ester (PFP), provided the corresponding tetrapeptide **18** in acceptable yield. Subsequent saponification gave rise to the simplified tubulysin derivative **19**.

Because the yields in the coupling step could not be improved, we also investigated an alternative order of peptide bond formation. Cleavage of the Z protecting group and subsequent peptide coupling with protected D-Pip gave rise to the tripeptide **20**, which could be saponified to afford the free acid **21** in quantitative yield.

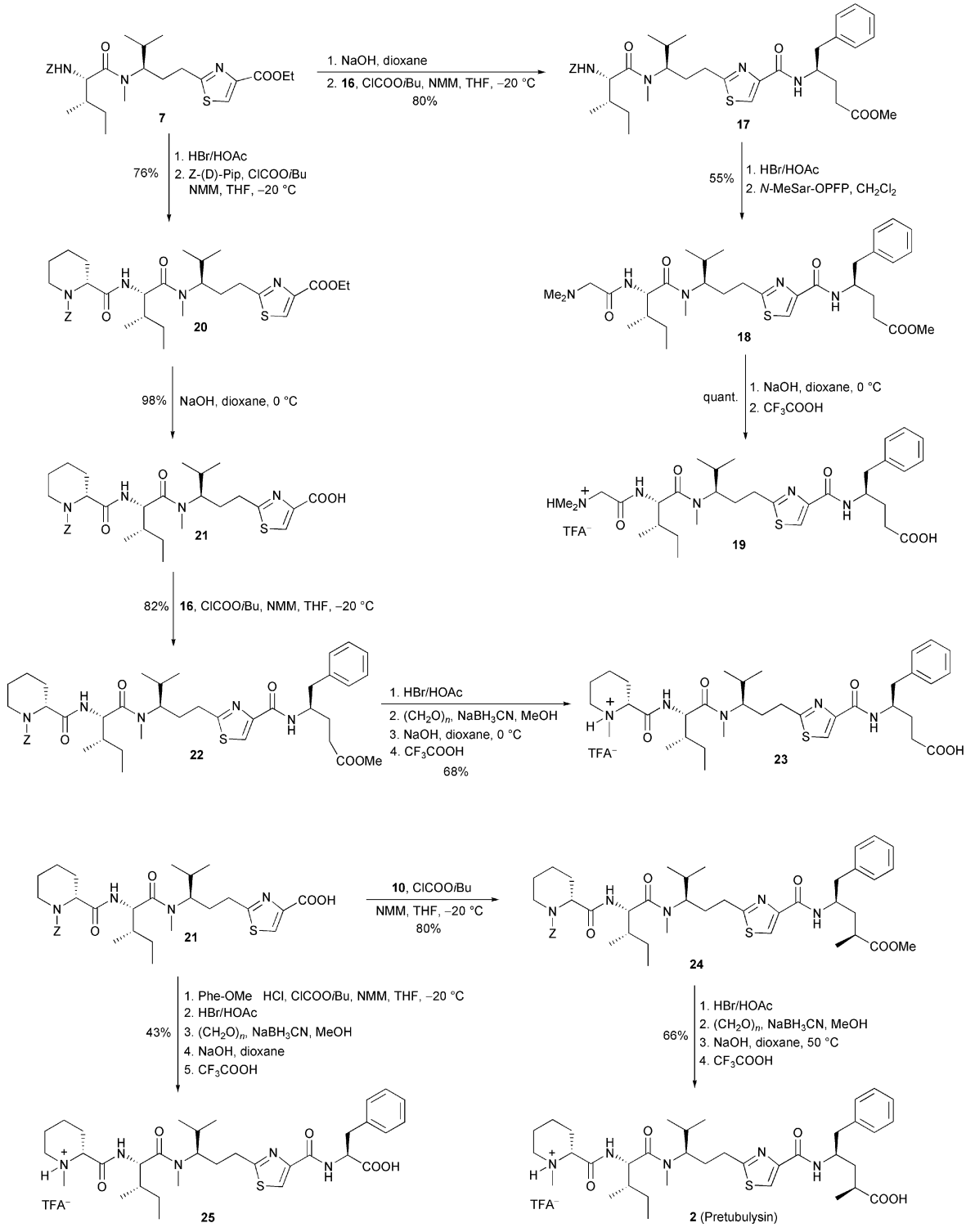
Coupling with dTup again proceeded without difficulty to give the tetrapeptide **22**. The sequence was completed by cleavage of the Z protecting group and reductive methylation of the pipecolic acid. Finally, saponification and acidification gave rise to demethylpretubulysin (**23**).

With this route to hand, the synthesis of pretubulysin (**2**) was accomplished in an analogous manner (Scheme 4). In view of SAR studies by Wipf[6] and Ellman,[7] indicating a high degree of variability at the tubulysin C-terminus, we also coupled **21** with Phe to determine whether or not the shortened compound **25** might retain good biological activity.

Pretubulysin (**2**) and its synthetic analogues **19**, **23**, and **25** were evaluated for their cytotoxicities relative to tubulysins A (**1A**) and D (**1D**), by measurement of their inhibition of cell growth by an MTT assay (Table 1). Tubulysin D is known to be the more toxic of the two metabolites, with a potency approximately six times higher than that of tubulysin A.[3] Human acute myeloid leukemia cells (HL-60) were selected for initial activity screening, because in our hands they are reproducibly sensitive relative to other cell lines. As predicted from the earlier SAR studies, pretubulysin (**2**) retained good activity relative to tubulysins A and D (cytotoxicity three and five times lower, respectively). However, all of the other analogues were less active. Removal of the C2-methyl group as in compound **23** led to a 13-fold relative reduction in cytotoxicity, whereas compound **19**, in which the *N*-terminal Mep had been replaced by a *N*-methylsarcosine unit, was essentially inactive (IC_{50}: 620 ng mL^{-1}). Derivative **25**, in which the Tup had been replaced by Phe, was similarly affected (IC_{50}: 790 ng mL^{-1}).

In view of these results, tubulysins A and D and compounds **2** and **23** were evaluated against a wide range of other tumor cell lines. Both **2** and **23** exhibited activity against all cell lines in the low- or subnanomolar range, albeit reduced relative to tubulysins A and D, with **2** being reproducibly the more potent. On average, the demethyl derivative **23** is less active by a factor of 10, although a higher activity was observed towards epithelial carcinoma cells (A431).

These results reveal several important SAR relationships, in addition to the data reported by other groups.[6–8] Notably, the good potency of pretubulysin (**2**) confirms that neither the *N*,*O*-acetal nor the acetoxy functionality of Tuv are necessary for cytotoxicity, although there is a modest reduction in activity (ca. 10-fold) relative to a previously characterized analogue in which the acetoxy group was retained.[6b] Comparison of the data obtained for **2** and its C2-demethyl analogue **23** reveals that the methyl group in Tup also provides an approximately 10-fold enhancement in biological activity. It has previously been demonstrated that the N-terminal Mep in tubulysin D can be replaced by



Scheme 4. Synthesis of pretubulysin and simplified derivatives.

a sarcosine unit with essentially full retention of bioactivity.[7] However, this modification in combination with the loss of the C2-methyl and both labile sites as in compound **19** resulted in a drastic (five orders of magnitude) decrease in activity. A similar drop was observed for the phenylalanine derivative **25**, suggesting the importance of this portion of the molecule in the absence of the acetoxy and *N*,*O*-acetal functionalities.

Table 1. Cytotoxicity of tubulysin, pretubulysin, and analogues towards a wide range of tumor cell lines.

Cell line	Origin	IC_{50} (ng mL^{-1})[a]			
		1A	**1D**	**2**	**23**
HL-60	acute promyelocytic leukemia[b]	0.05	0.003	0.014	0.22
A431	epithelial carcinoma[b]	0.10	0.012	1.24	0.34
A549	lung carcinoma[b]	0.09	0.006	4.35	31.2
COS-7	kidney carcinoma[c]	0.08	0.034	1.13	9.63
HEK293T	kidney[b]	0.05	0.010	0.23	1.68
HepG2	hepatocell. carcinoma[b]	0.12	0.007	0.64	8.75
K562	chronic myelogenous leukemia[b]	0.40	0.022	0.35	2.50
KB3.1	cervical carcinoma[b]	0.16	0.018	2.32	26.3
L929	subcutaneous connective tissue[d]	0.21	0.017	3.87	40.0
SH-SY5Y	neuroblastoma[b]	0.21	0.011	0.61	2.90
SW480	colon adenocarcinoma[b]	0.02	0.004	0.21	3.16
U-2 OS	bone osteosarcoma[b]	0.19	0.034	0.56	3.23
U937	histiocytic lymphoma[b]	0.05	0.003	0.04	0.38

[a] Values each represent the average of two measurements. Incubation time: 5 d. [b] Human. [c] African green monkey. [d] Mouse.

Conclusions

In conclusion, we have shown that the complex structures of the tubulysins can be significantly reduced without dramatic drops in their biological activities. Pretubulysin (**2**), which was identified as direct biosynthetic precursor of the tubulysins (**1**), has an activity similar to or slightly less than that of tubulysin A (**1A**) (1–50-fold), but retains low- or sub-nM activity. Taken together, these findings should aid in future efforts to design simplified, yet highly potent analogues of the tubulysins for evaluation as anticancer agents.

Experimental Section

General Remarks: Reactions with dry solvents were carried out in oven-dried glassware (100 °C) under nitrogen. Solvents were dried as follows: THF was distilled from $LiAlH_4$, CH_2Cl_2 from CaH_2, MeOH from Mg, and toluene from Na. The products were purified by flash chromatography on silica gel (0.063–0.2 mm). Mixtures of ethyl acetate (EtOAc) and hexane were generally used as eluents. Analysis by TLC was carried out with commercially precoated Polygram SIL-G/UV 254 plates (Machery–Nagel, Dueren). Visualization was accomplished with the aid of UV light, $KMnO_4$ solution, or iodine. ^{1}H NMR and ^{13}C NMR spectra were obtained at room temperature with Bruker AV 400 and AV 500 spectrometers. Chemical shifts are expressed in ppm relative to internal solvent. Asterisked signals are estimated values from ^{13}C- or HSQC-spectra. Selected signals of minor isomers are extracted from the NMR spectra of the isomeric mixtures. The enantiomeric and diastereomeric ratios were determined by HPLC with a Shimadzu 10A VP with chiral columns (Reprosil 100 Chiral-NR 8 μm, Chiralcel OD-H) or an achiral silica gel column (Lichrosorb 5 μ Si-60 A). Optical rotation measurements were performed with a Perkin–Elmer 341 polarimeter, with concentrations given in g/100 mL. Melting points were determined with a MEL-TEMP II apparatus and are uncorrected. High-resolution mass spectra were recorded with a Finnigan MAT 95Q instrument by the CI technique. Elemental analyses were performed with a Leco CHN900 instrument.

General Procedure for in-situ DibalH Reduction/Wittig Reaction: A solution of DibalH in hexane (1 M, 2.0 equiv.) was slowly added dropwise at –78 °C to a solution of a Boc-protected amino acid methyl ester (1.0 equiv.) in dry toluene or dichloromethane (3 mL mmol^{-1}). After the system had been stirred for 30 min at –78 °C, the phosphonium salt (2.0 equiv.) and KO*t*Bu (2.0 equiv.), suspended in dry toluene or dichloromethane (2 mL mmol^{-1}), were added to the reaction mixture. The cooling bath was removed after 1 h, and the reaction mixture was stirred overnight at room temperature, poured into saturated potassium sodium tartrate (15 mL mmol^{-1}), and vigorously stirred for 30 min. The aqueous layer was extracted three times with EtOAc, and the combined organic layers were dried with Na_2SO_4. Purification by flash chromatography provided the elongated unsaturated amino acid derivative.

(4*S*,2*E*)-4-(*tert*-Butoxycarbonylamino)-5-methylhex-2-enenitrile (3): The nitrile **3** was obtained from Boc-L-valine methyl ester (1.74 g, 7.52 mmol), DibalH (1 M solution in hexane, 15 mL, 15 mmol), (cyanomethyl)triphenylphosphonium chloride (4.67 g, 13.8 mmol), and KO*t*Bu (1.55 g, 13.8 mmol) by the General Procedure for in situ DibalH reduction/Wittig reaction as a white solid (1.20 g, 5.35 mmol, 71%, >99% *ee*) after flash chromatography (hexane/EtOAc, 1. 9:1, 2. 8:2). R_f = 0.29 (Hex/EtOAc, 8:2). HPLC: Reprosil 100 Chiral-NR 8 μm, Hex/*i*PrOH, 95:5, 2 mL min^{-1}, t_R[(*R*)-**3**] = 5.91 min, t_R[(*S*)-**3**] = 6.94 min; m.p. 62 °C. $[\alpha]_D^{20}$ = –5.7 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.90 (d, $^3J_{6,5}$ = 6.7 Hz, 3 H, 6-H), 0.93 (d, $^3J_{6',5}$ = 6.7 Hz, 3 H, 6′-H), 1.43 (s, 9 H, 9-H), 1.84 (m, 1 H, 5-H), 4.11 (br. s, 1 H, 4-H), 4.50 (br. s, 1 H, NH), 5.47 (dd, $^3J_{2,3}$ = 16.5, $^4J_{2,4}$ = 1.6 Hz, 1 H, 2-H), 6.62 (dd, $^3J_{3,2}$ = 16.5, $^3J_{3,4}$ = 5.5 Hz, 1 H, 3-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.0, 18.8, 28.3, 32.0, 57.4, 80.2, 100.4, 117.1, 154.0, 155.1 ppm. HRMS (CI): *m*/*z* calcd. for $C_{12}H_{21}N_2O_2$ [M + H]$^+$ 225.1603; found 225.1564. $C_{12}H_{20}N_2O_2$ (224.30): calcd. C 64.26, H 8.99, N 12.49; found C 64.04, H 9.25, N 12.86.

(4*R*)-4-(*tert*-Butoxycarbonyl-methylamino)-5-methylhexanenitrile (4): A solution of **3** (2.77 g, 12.3 mmol) in MeOH (40 mL) was stirred under hydrogen in the presence of Pd/C (10%, 138 mg) until reduction was complete. After filtration through celite, purification by flash chromatography (hexane/EtOAc, 8:2) gave the saturated nitrile as a white solid in 87% yield (2.41 g, 10.6 mmol). A solution of this nitrile (2.39 g, 10.6 mmol) and methyl iodide (2.6 mL, 41.8 mmol) in dry DMF (40 mL) was treated at 0 °C with a suspension of NaH (60%, 964 mg, 24.1 mmol) in dry DMF (10 mL). The reaction mixture was allowed to warm to room temperature overnight. After dilution with water (200 mL) and saturated NH_4Cl (50 mL), the solution was extracted three times with EtOAc, and the combined organic layers were dried with Na_2SO_4. The crude product was purified by flash chromatography (hexane/EtOAc, 8:2) to afford **4** (2.51 g, 10.4 mmol, 98%) as a colorless oil. R_f = 0.18 (Hex/EtOAc, 8:2). $[\alpha]_D^{20}$ = +7.0 (*c* = 1.0, $CHCl_3$). ^{1}H NMR analysis at room temperature showed a 1:1 mixture of rotamers: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.83 (d, $^3J_{6,5}$ = 7.0 Hz, 3 H, 6-H), 0.85 (d, $^3J_{6,5}$ = 6.8 Hz, 3 H, 6-H), 0.93 (d, $^3J_{6',5}$ = 6.5 Hz, 3 H, 6′-H), 0.94 (d, $^3J_{6',5}$ = 6.5 Hz, 3 H, 6′-H), 1.43 (s, 9 H, 10-H), 1.45 (s, 9 H, 10-H), 1.62–1.75 (m, 4 H, 3-H$_a$, 5-H), 1.91–2.03 (m, 2 H, 3-H$_b$), 2.12–2.34 (m, 4 H, 2-H$_a$, 2-H$_b$), 2.64 (s, 6 H, 7-H), 3.62 (br. s, 1 H, 4-H), 3.68 (ddd, $^3J_{4,3a/b} \approx {}^3J_{4,5}$ = 10.8, $^3J_{4,3a/b}$ = 3.3 Hz, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.5, 19.6, 19.8, 20.0, 20.1, 25.9, 28.4, 28.8, 30.3, 30.5, 60.8, 61.1, 79.7, 80.2, 119.3, 119.7, 156.3, 156.6 ppm. HRMS (CI): *m*/*z* calcd. for $C_{13}H_{25}N_2O_2$ [M + H]$^+$ 241.1916; found 241.1922. $C_{13}H_{24}N_2O_2$ (240.35): calcd. C 64.97, H 10.06, N 11.66; found C 65.14, H 9.91, N 11.50.

(4*R*)-4-[(*tert*-Butoxycarbonyl)methylamino]-5-methylhexanethioamide (5): Hydrogen sulfide was passed at –78 °C for 1 h through a solution of nitrile **4** (1.85 g, 7.70 mmol) and triethylamine (5.4 mL, 38.4 mmol) in chloroform (15 mL).[11] The flask was sealed and the reaction mixture was allowed to warm to room temperature overnight. After stirring for 5 d at room temperature the solution was washed with HCl (1 M), saturated $NaHCO_3$, and water. After drying of the organic phase over Na_2SO_4 and evaporation of the solvent in vacuo, the thioamide **5** (1.94 g, 7.07 mmol, 92%) was obtained after flash chromatography (hexane/EtOAc, 1:1) as a white solid. R_f = 0.33 (Hex/EtOAc, 1:1). $[\alpha]_D^{20}$ = +20.7 (*c* = 1.0, $CHCl_3$). Major rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82 (d, $^3J_{6,5}$ = 6.5 Hz, 3 H, 6-H), 0.93 (d, $^3J_{6',5}$ = 6.8 Hz, 3 H, 6′-H), 1.44 (s, 9 H, 10-H), 1.60 (m, 1 H, 5-H), 1.70 (dddd, $^2J_{3a,3b}$ = 14.6, $^3J_{3a,4}$ = 12.3, $^3J_{3a,2b}$ = 6.8, $^3J_{3a,2a}$ = 3.5 Hz, 1 H, 3-H$_a$), 2.04 (dddd, $^2J_{3b,3a}$ = 14.6, $^3J_{3b,2a}$ = 11.0, $^3J_{3b,2b} \approx {}^3J_{3b,4}$ = 3.0 Hz, 1 H, 3-H$_b$), 2.39 (ddd, $^2J_{2a,2b}$ = 12.6, $^3J_{2a,3b}$ = 11.0, $^3J_{2a,3a}$ = 3.5 Hz, 1 H, 2-H$_a$), 2.61 (s, 3 H, 7-H), 2.73 (dddd, $^2J_{2b,2a}$ = 12.6, $^3J_{2b,3a}$ = 6.8, $^3J_{2b,3b}$ = 3.5, $^4J_{2b,4}$ = 1.5 Hz, 1 H, 2-H$_b$), 3.68 (ddd, $^3J_{4,3a}$ = 12.3, $^3J_{4,5}$ = 10.3, $^3J_{4,3b}$ = 2.5 Hz, 1 H, 4-H), 7.48 (br. s, 1 H, NH), 8.71 (br. s, 1 H, NH′) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.9, 20.2, 27.9, 28.4, 28.7, 29.8, 41.9, 60.0, 80.2, 158.0, 210.4 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42 (s, 9 H, 10-H), 2.24 (m, 1 H, 3-H$_b$), 2.43–2.57 (m, 2 H, 2-H$_a$, 2-H$_b$), 2.65 (s, 3 H, 7-H), 3.61 (br. s, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.9, 28.5, 41.5, 79.8, 153.3, 211.6 ppm. HRMS (CI): *m*/*z* calcd. for $C_{13}H_{27}N_2O_2S$ [M + H]$^+$ 275.1793; found 275.1769. $C_{13}H_{26}N_2O_2S$ (274.42): calcd. C 56.90, H 9.55, N 10.21; found C 57.04, H 9.25, N 10.10.

Ethyl (*R*)-2-{3-[(*tert*-Butoxycarbonyl)methylamino]-4-methylpentyl}-thiazole-4-carboxylate (6): Ethyl bromopyruvate (90%, 770 μL, 5.52 mmol) was added dropwise at –10 °C to a solution of thioamide **5** (1.37 g, 4.99 mmol) in dry acetone (7.5 mL).[12] After 1.5 h the reaction mixture was poured into a vigorously stirred mixture of dichloromethane (25 mL) and saturated $KHCO_3$ (25 mL). The aqueous layer was extracted with dichloromethane, and the solvent was evaporated in vacuo after drying of the combined organic layers over Na_2SO_4. Pyridine (890 μL, 11.0 mmol) was added at –20 °C to a solution of the hydroxythiazoline intermediate in dry dichloromethane (6 mL), and trifluoroacetic anhydride (1.05 mL, 5.49 mmol) was added dropwise. The reaction mixture was allowed to warm to 0 °C over 2 h and was stirred overnight at room temperature. The solution was diluted with dichloromethane (10 mL) and washed twice with saturated $NaHCO_3$. The aqueous layers were extracted with dichloromethane, and the combined organic layers were washed with $KHSO_4$ (1 M) and dried with Na_2SO_4. The crude product was purified by flash chromatography (hexane/EtOAc, 8:2, 7:3, 1:1) to afford the thiazole **6** (1.56 g, 4.22 mmol, 84%, >99% *ee*) as a pale yellow oil. R_f = 0.23 (Hex/EtOAc, 7:3). HPLC: Chiralcel OD-H, Hex/*i*PrOH, 9:1, 1 mL min^{-1}, t_R[(*S*)-**6**] = 6.91 min, t_R[(*R*)-**6**] = 9.12 min. $[\alpha]_D^{20}$ = –13.3 (*c* = 1.0, $CHCl_3$). ^{1}H NMR analysis at room temperature showed a 54:46 mixture of rotamers; major rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.83 (d, $^3J_{9,8}$ = 6.8 Hz, 3 H, 9-H), 0.93 (d, $^3J_{9',8}$ = 6.5 Hz, 3 H, 9′-H), 1.38 (t, $^3J_{15,14}$ = 7.2 Hz, 3 H, 15-H), 1.44 (s, 9 H, 13-H), 1.65 (m, 1 H, 8-H), 1.82 (m, 1 H, 6-H$_a$), 2.12 (m, 1 H, 6-H$_b$), 2.63 (s, 3 H, 10-H), 2.95 (t, $^3J_{5,6}$ = 8.1 Hz, 2 H, 5-H), 3.82 (m, 1 H, 7-H), 4.40 (q, $^3J_{14,15}$ = 7.2 Hz, 2 H, 14-H), 8.03 (s, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.4, 19.6, 19.9, 28.2, 28.5, 30.0, 30.5, 30.7, 60.3, 61.4, 79.6, 128.6, 147.0, 156.6, 161.4, 171.7 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.94 (d, $^3J_{9',8}$ = 6.8 Hz, 3 H, 9′-H), 1.38 (t, $^3J_{15,14}$ = 7.0 Hz, 3 H, 15-H), 1.41 (s, 9 H, 13-H), 2.68 (s, 3 H, 10-H), 2.94 (t, $^3J_{5,6}$ = 7.9 Hz, 2 H, 5-H), 3.63 (br. s, 1 H, 7-H), 4.39 (q, $^3J_{14,15}$ = 7.0 Hz, 2 H, 14-H), 8.01 (s, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.1, 20.3, 30.1, 30.7, 30.8, 61.4, 79.2, 126.9, 146.8, 156.4, 161.5, 171.4 ppm. HRMS (CI): *m*/*z* calcd. for $C_{18}H_{31}N_2O_4S$ [M + H]$^+$ 371.2004; found 371.1964.

Ethyl (*R*)-2-{3-[(*N*-Benzyloxycarbonyl-(*S*)-isoleucyl)methylamino]-4-methylpentyl}thiazole-4-carboxylate (7): A solution of HCl in dioxane (4 M, 7.5 mL, 30 mmol) was added at 0 °C to thiazole **6** (1.11 g, 3.00 mmol). The solvent was evaporated in vacuo after complete deprotection (TLC monitoring) and the hydrochloride salt was dried in high vacuum. *N*-Deprotected **6**, Z-protected L-isoleucine (876 mg, 3.30 mmol), and 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP, 905 mg, 3.30 mmol) were dissolved in dry dichloromethane (30 mL) and diisopropyl ethylamine (2.04 mL, 12 mmol) was added dropwise to this solution at –10 °C.[13] The cooling bath was removed after 20 min and the reaction mixture was stirred at room temperature overnight. The dipeptide **7** (1.42 g, 2.64 mmol, 88%) was obtained after flash chromatography (hexane/EtOAc, 1. 7:3, 2. 1:1) as a yellow oil. R_f = 0.34 (Hex/EtOAc, 1:1). $[\alpha]_D^{20}$ = –22.9 (*c* = 1.0, $CHCl_3$). Major rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.75 (d, $^3J_{9,8}$ = 6.5 Hz, 3 H, 9-H), 0.86 (t, $^3J_{15,14}$ = 7.4 Hz, 3 H, 15-H), 0.95 (d, $^3J_{16,13}$ = 6.5 Hz, 3 H, 16-H), 0.96 (d, $^3J_{9',8}$ = 6.5 Hz, 3 H, 9′-H), 1.11 (ddq, $^2J_{14a,14b}$ = 13.6, $^3J_{14a,13}$ = 9.7, $^3J_{14a,15}$ = 7.3 Hz, 1 H, 14-H$_a$), 1.37 (t, $^3J_{24,23}$ = 7.0 Hz, 3 H, 24-H), 1.57 (dqd, $^2J_{14b,14a}$ = 13.6, $^3J_{14b,15}$ = 7.6, $^3J_{14b,13}$ = 3.0 Hz, 1 H, 14-H$_b$), 1.63–1.79 (m, 2 H, 8-H, 13-H), 1.88 (m, 1 H, 6-H$_a$), 2.14 (m, 1 H, 6-H$_b$), 2.83 (dt, $^2J_{5a,5b}$ = 15.2, $^3J_{5a,6}$ = 6.3 Hz, 1 H, 5-H$_a$), 2.89 (dt, $^2J_{5b,5a}$ = 15.2, $^3J_{5b,6}$ = 6.3 Hz, 1 H, 5-H$_b$), 2.94 (s, 3 H, 10-H), 4.32 (m, 1 H, 7-H), 4.39 (q, $^3J_{23,24}$ = 7.0 Hz, 1 H, 23-H), 4.52 (dd, $^3J_{12,NH}$ = 9.5, $^3J_{12,13}$ = 6.8 Hz, 1 H, 12-H), 5.05 (d, $^2J_{18a,18b}$ = 12.4 Hz, 1 H, 18-H$_a$), 5.08 (d, $^2J_{18b,18a}$ = 12.4 Hz, 1 H, 18-H$_b$), 5.40 (d, $^3J_{NH,12}$ = 9.5 Hz, 1 H, NH$_{Ile}$), 7.18–7.36 (m, 5-H, arom. H), 8.01 (s, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2, 14.3, 16.0, 19.5, 20.0, 23.8, 29.4, 29.7, 30.1, 30.6, 37.4, 55.7, 59.1, 61.3, 66.7, 126.9, 127.8, 128.0, 128.4, 136.4, 146.9, 156.4, 161.3, 170.9, 173.3 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.04 (d, $^3J_{9',8}$ = 6.8 Hz, 3 H, 9′-H), 2.23 (m, 1 H, 6-H$_a$), 2.74 (s, 3 H, 10-H), 3.58 (ddd, $^3J_{7,6a/b} \approx {}^3J_{7,8}$ = 10.2, $^3J_{7,6a/b}$ = 3.3 Hz, 1 H, 7-H), 4.57 (dd, $^3J_{12,NH}$ = 9.6, $^3J_{12,13}$ = 6.3 Hz, 1 H, 12-H), 7.98 (s, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.3, 16.1, 20.3, 20.4, 23.5, 27.3, 29.8, 31.3, 37.8, 55.2, 62.6, 66.9, 128.4, 136.3, 156.2, 170.5, 172.5 ppm. HRMS (CI): *m*/*z* calcd. for $C_{27}H_{40}N_3O_5S$ [M + H]$^+$ 518.2689; found 518.2721. $C_{27}H_{39}N_3O_5S$ (517.68): calcd. C 62.64, H 7.59, N 8.12; found C 62.30, H 7.61, N 8.24.

Ethyl (4*S*,2*E*)-4-[(*tert*-Butoxycarbonyl)amino]-2-methyl-5-phenylpent-2-enoate (8):[12b,18] The unsaturated ester **8** was obtained as a white solid (4.78 g, 14.3 mmol, 82%, >99% *ee*) from Boc-L-phenylalanine methyl ester (4.90 g, 17.5 mmol), DibalH (1 M in hexane, 35 mL, 35 mmol), (1-ethoxycarbonyl-ethyl)-triphenylphosphonium bromide (15.5 g, 35.0 mmol), and KO*t*Bu (4.03 g, 35.9 mmol) by the General Procedure for in situ DibalH reduction/Wittig reaction and flash chromatography (hexane/EtOAc, 95:5, 9:1). R_f = 0.31 (Hex/EtOAc, 8:2). HPLC: Reprosil 100 Chiral-NR 8 μm, Hex/*i*PrOH, 8:2, 2 mL min^{-1}, t_R[(*R*)-**8**] = 5.96 min, t_R[(*S*)-**8**] = 6.86 min; m.p. 68 °C. $[\alpha]_D^{20}$ = +33.0 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.26 (t, $^3J_{15,14}$ = 7.3 Hz, 3 H, 15-H), 1.38 (s, 9 H, 13-H), 1.68 (d, $^4J_{10,3}$ = 1.5 Hz, 3 H, 10-H), 2.76 (dd, $^2J_{5a,5b}$ = 13.3, $^3J_{5a,4}$ = 7.0 Hz, 1 H, 5-H$_a$), 2.90 (m, 1 H, 5-H$_b$), 4.16 (q, $^3J_{14,15}$ = 7.3 Hz, 2 H, 14-H), 4.55 (br. s, 1 H, NH), 4.64 (br. s, 1 H, 4-H), 6.49 (dd, $^3J_{3,4}$ = 9.1, $^4J_{3,10}$ = 1.5 Hz, 1 H, 3-H), 7.15 (d, $^3J_{7,8}$ = 7.0 Hz, 2 H, 7-H), 7.20 (t, $^3J_{9,8}$ = 7.3 Hz, 1 H, 9-H), 7.26 (dd, $^3J_{8,7} \approx {}^3J_{8,9}$ = 7.2 Hz, 2 H, 8-H) ppm. ^{13}C NMR (100 MHz,

$CDCl_3$): δ = 12.5, 14.2, 28.3, 41.1, 50.1, 60.6, 79.6, 126.6, 128.4, 129.2, 129.5, 136.7, 140.2, 154.9, 167.7 ppm.

Ethyl (4*S*)-4-[(*tert*-Butoxycarbonyl)amino]-2-methyl-5-phenylpentanoate (9): A solution of **8** (219 mg, 0.657 mmol) in MeOH (8 mL) was stirred under hydrogen with Pd/C (10%, 20 mg) until no double bond could be detected (TLC), after which the catalyst was filtered off through celite. Purification by flash chromatography (hexane/EtOAc, 9:1) afforded **9** as a colorless oil (195 mg, 0.581 mmol, 88%, *dr* 2:1). R_f = 0.29 (Hex/EtOAc, 8:2). Major diastereomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.12 (d, $^3J_{10,2}$ = 7.3 Hz, 3 H, 10-H), 1.21 (t, $^3J_{15,14}$ = 7.2 Hz, 3 H, 15-H), 1.37 (s, 9 H, 13-H), 1.40 (m, 1 H, 3-H_a), 1.86 (ddd, $^2J_{3b,3a}$ = 13.3, $^3J_{3b,2/4}$ = 9.7, $^3J_{3b,4/2}$ = 3.7 Hz, 1 H, 3-H_b), 2.55 (m, 1 H, 2-H), 2.75 (d, $^3J_{5,4}$ = 5.7 Hz, 1 H, 5-H), 3.85 (m, 1 H, 4-H), 4.09 (q, $^3J_{14,15}$ = 7.0 Hz, 2 H, 14-H), 4.30 (d, $^3J_{NH,4}$ = 8.8 Hz, 1 H, NH), 7.15 (d, $^3J_{7,8}$ = 7.3 Hz, 2 H, 7-H), 7.18 (t, $^3J_{9,8}$ = 7.3 Hz, 1 H, 9-H), 7.26 (dd, $^3J_{8,7}$ = $^3J_{8,9}$ = 7.3 Hz, 2 H, 8-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.2, 17.6, 28.4, 36.4, 37.9, 41.4, 49.8, 60.4, 79.0, 126.3, 128.3, 129.5, 137.9, 155.1, 176.2 ppm. Selected signals of the minor diastereomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.10 (d, $^3J_{10,2}$ = 7.3 Hz, 3 H, 10-H), 1.22 (t, $^3J_{15,14}$ = 7.2 Hz, 3 H, 15-H), 1.49 (ddd, $^2J_{3a,3b}$ = 14.1, $^3J_{3a,2/4}$ = 7.3, $^3J_{3a,4/2}$ = 3.7 Hz, 1 H, 3-H_a), 1.71 (m, 1 H, 3-H_b), 2.44 (m, 1 H, 2-H), 2.69 (dd, $^2J_{5a,5b}$ = 13.6, $^3J_{5a,4}$ = 7.0 Hz, 1 H, 5-H_a), 2.79 (dd, $^2J_{5b,5a}$ = 13.6, $^3J_{5b,4}$ = 5.7 Hz, 1 H, 5-H_b), 3.75 (m, 1 H, 4-H), 4.10 (q, $^3J_{14,15}$ = 7.0 Hz, 2 H, 14-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.2, 17.3, 37.0, 37.6, 42.4, 50.3, 60.4, 155.3 ppm. HRMS (CI): *m*/*z* calcd. for $C_{19}H_{30}NO_4$ $[M + H]^+$ 336.2175; found 336.2187.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-4-methyl-1-phenylpentan-2-amine (10):[19] A solution of DibalH in hexane (1 M, 1.3 mL, 1.3 mmol) was added dropwise at 0 °C to a solution of ester **8** (136 mg, 0.41 mmol) in dichloromethane (2.5 mL). After 2.5 h the reaction mixture was poured into saturated potassium sodium tartrate and stirred vigorously. The aqueous layer was extracted three times with dichloromethane and the combined organic layers were dried with Na_2SO_4. The allyl alcohol was obtained by flash chromatography (hexane/EtOAc, 7:3, 6:4) as a white solid (83 mg, 0.285 mmol, 70%). A solution of this allyl alcohol (69 mg, 0.237 mmol) in MeOH (2.8 mL) was stirred under hydrogen with Pd/C (10%, 7 mg) until complete hydrogenation (TLC) and the catalyst was filtered off through a pad of celite. Purification by flash chromatography (hexane/EtOAc, 8:2) afforded the amide **10** as a white solid (58 mg, 0.209 mmol, 88%). R_f = 0.60 (Hex/EtOAc, 1:1); m.p. 115 °C. $[\alpha]_D^{20}$ = +36.1 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.85 (d, $^3J_{1,2}$ = 6.6 Hz, 3 H, 1-H), 0.87 (d, $^3J_{1',2}$ = 7.0 Hz, 3 H, 1′-H), 1.22 (dd, $^3J_{3,2/4}$ = 7.3, $^3J_{3,4/2}$ = 7.0 Hz, 2 H, 3-H), 1.38 (s, 9 H, 12-H), 1.66 (m, 1 H, 2-H), 2.73 (m, 2 H, 5-H), 3.77 (m, 1 H, 4-H), 4.22 (br. s, 1 H, NH), 7.15 (d, $^3J_{7,8}$ = 7.3 Hz, 2 H, 7-H), 7.18 (t, $^3J_{9,8}$ = 7.3 Hz, 1 H, 9-H), 7.26 (dd, $^3J_{8,7}$ = $^3J_{8,9}$ = 7.3 Hz, 2 H, 8-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 23.2, 24.8, 28.4, 41.8, 43.6, 49.6, 78.9, 126.2, 128.2, 129.6, 138.3, 155.4 ppm.

(*R*)-5-{2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropyl}-2,2-dimethyl-1,3-dioxane-4,6-dione (11):[17] As described in the literature, Boc-L-phenylalanine (5.32 g, 20.1 mmol) and Meldrum's acid[16] (3.21 g, 22.3 mmol) were coupled with the aid of dicyclohexyl carbodiimide (4.76 g, 23.1 mmol) and DMAP (3.95 g, 32.3 mmol) in dichloromethane (150 mL). The intermediate was reduced with sodium borohydride (1.90 g, 50.2 mmol) and acetic acid (13.5 mL, 236 mmol) to give **11** as a white solid (4.35 g, 9.66 mmol, 48%) after column chromatography (hexane/EtOAc, 8:2). R_f = 0.49 (Hex/EtOAc, 1:1); m.p. 111–113 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.33 (s, 9 H, 12-H), 1.71 (s, 3 H, 14-H), 1.75 (s, 3 H, 14′-H), 2.13 (m, 1 H, 5-H_a), 2.26 (m, 1 H, 5-H_b), 2.84 (d, $^3J_{3,4}$ = 6.0 Hz, 2 H, 3-H), 3.89 (br. s, 1 H, 2-H), 4.21 (m, 1 H, 4-H), 4.44 (br. s, 1 H, NH), 7.15–7.23 (m, 3 H, 7-H, 9-H), 7.28 (dd, $^3J_{8,7}$ = $^3J_{8,9}$ = 7.3 Hz, 2 H, 8-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.0, 28.2, 28.6, 31.4, 41.8, 44.3, 49.9, 79.6, 105.0, 126.7, 128.6, 129.3, 137.1, 156.7, 165.5, 165.7 ppm.

Methyl (*S*)-4-[(*tert*-Butoxycarbonyl)amino]-2-methylene-5-phenylpentanoate (12):[20] As described in the literature, **12** was obtained from **11** (250 mg, 0.662 mmol) and *N*,*N*-dimethylmethyleneammonium iodide (308 mg, 1.66 mmol) in dry MeOH (8 mL) as white solid (169 mg, 0.529 mmol, 80%) after column chromatography (hexane/EtOAc, 8:2). R_f = 0.29 (Hex/EtOAc, 8:2); m.p. 102 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.36 (s, 9 H, 13-H), 2.30 (m, 1 H, 3-H_a), 2.53 (dd, $^2J_{3b,3a}$ = 14.1, $^3J_{3b,4}$ = 4.2 Hz, 1 H, 3-H_b), 2.75 (dd, $^2J_{5a,5b}$ = 13.6, $^3J_{5a,4}$ = 6.8 Hz, 1 H, 5-H_a), 2.86 (m, 1 H, 5-H_b), 3.73 (s, 3 H, 14-H), 3.99 (m, 1 H, 4-H), 4.44 (br. s, 1 H, NH), 5.56 (d, $^2J_{10a,10b}$ = 1.0 Hz, 1 H, 10-H_a), 6.19 (d, $^2J_{10b,10a}$ = 1.0 Hz, 1 H, 10-H_b), 7.15–7.23 (m, 3 H, 7-H, 9-H), 7.28 (dddd, $^3J_{8,7}$ = $^3J_{8,9}$ = 7.2, $^4J_{8,8'}$ = $^5J_{8,7'}$ = 1.4 Hz, 2 H, 8-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.3, 36.5, 41.3, 51.3, 51.9, 79.0, 126.4, 127.4, 128.4, 129.4, 137.3, 138.0, 155.3, 167.7 ppm.

(1*R*,2*S*,5*R*)-(–)-Menthyl (4*S*,2*E*)-4-[(*tert*-Butoxycarbonyl)amino]-2-methyl-5-phenylpent-2-enoate (13): A mixture of ethyl ester **8** (668 mg, 2.00 mmol) and NaOH (1 M, 2.4 mL, 2.4 mmol) in dioxane (20 mL) was heated to 80 °C for 2 h. The solvent was evaporated in vacuo, and the residue was dissolved in water. After extraction with EtOAc, and discarding of the organic layer the aqueous layer was acidified to pH 2 with HCl (1 M) and extracted twice with EtOAc. The combined organic layers were dried with Na_2SO_4 and the solvent was evaporated in vacuo to give the corresponding acid (582 mg, 1.91 mmol, 95%) as a white solid. A solution of DCC (483 mg, 2.34 mmol) in diethyl ether (3 mL) was added at 0 °C to a solution of this acid (657 mg, 2.15 mmol), (–)-menthol (840 mg, 5.38 mmol), and DMAP (26 mg, 0.213 mmol) in diethyl ether (21.5 mmol). The reaction mixture was allowed to warm to room temperature overnight. After filtration of the precipitated urea the crude product was purified by flash chromatography (hexane/EtOAc, 95:5) to give the unsaturated menthyl ester **13** (690 mg, 1.56 mmol, 72%) as a white solid. R_f = 0.43 (Hex/EtOAc, 8:2); m.p. 89–90 °C. $[\alpha]_D^{20}$ = –27.8 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.74 (d, $^3J_{21,20}$ = 6.8 Hz, 3 H, 21-H), 0.85 [dddd, $^2J_{17ax,17eq} \approx {}^3J_{17ax,16ax} \approx {}^3J_{17ax,18(ax)}$ = 11.8, $^3J_{17ax,16eq}$ = 2.8 Hz, 1 H, 17-H_{ax}], 0.88 (d, $^3J_{22,18}$ = 6.3 Hz, 3 H, 22-H), 0.89 (d, $^3J_{21',20}$ = 6.8 Hz, 3 H, 21′-H), 0.93 [ddd, $^2J_{19ax,19eq} \approx {}^3J_{19ax,18ax} \approx {}^3J_{19ax,14(ax)}$ = 11.6 Hz, 1 H, 19-H_{ax}], 1.05 [dddd, $^2J_{16ax,16eq} \approx {}^3J_{16ax,15(ax)} \approx {}^3J_{16ax,17ax}$ = 12.9, $^3J_{16ax,17eq}$ = 3.0 Hz, 1 H, 16-H_{ax}], 1.39 [dddd, $^3J_{15(ax),14(ax)} \approx {}^3J_{15(ax),16ax}$ = 10.0, $^3J_{15(ax),16eq} \approx {}^3J_{15(ax),20}$ = 3.0 Hz, 1 H, 15-$H_{(ax)}$], 1.39 (s, 9 H, 13-H), 1.48 [m, 1 H, 18-$H_{(ax)}$], 1.63–1.71 (m, 2 H, 16-H_{eq}, 17-H_{eq}), 1.69 (d, $^4J_{10,3}$ = 1.5 Hz, 3 H, 10-H), 1.83 [qqd, $^3J_{20,21}$ = $^3J_{20,21'}$ = 6.8, $^3J_{20,15(ax)}$ = 2.8 Hz, 1 H, 20-H], 1.98 (m, 1 H, 19-H_{eq}), 2.77 (dd, $^2J_{5a,5b}$ = 13.3, $^3J_{5a,4}$ = 7.0 Hz, 1 H, 5-H_a), 2.91 (dd, $^2J_{5b,5a}$ = 13.3, $^3J_{5b,4}$ = 5.5 Hz, 1 H, 5-H_b), 4.54 (br. s, 1 H, NH), 4.64 (br. s, 1 H, 4-H), 4.68 [ddd, $^3J_{14(ax),15(ax)} \approx {}^3J_{14(ax),19ax}$ = 10.8, $^3J_{14(ax),19eq}$ = 4.5 Hz, 1 H, 14-$H_{(ax)}$], 6.44 (dd, $^3J_{3,4}$ = 9.2, $^4J_{3,10}$ = 1.5 Hz, 1 H, 3-H), 7.12 (m, 2 H, 7-H), 7.19 (tt, $^3J_{9,8}$ = 7.3, $^4J_{9,7}$ = 1.9 Hz, 1 H, 9-H), 7.25 (dddd, $^3J_{8,7} \approx {}^3J_{8,9}$ = 7.1, $^4J_{8,8'} \approx {}^5J_{8,7'}$ = 1.8 Hz, 2 H, 8-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.7, 16.4, 20.8, 22.0, 23.5, 26.4, 28.3, 31.4, 34.3, 40.9, 41.2, 47.2, 50.0, 74.5, 79.7, 126.7, 128.4, 129.6, 139.6, 154.9, 167.1 ppm. $C_{27}H_{41}NO_4$ (443.63): calcd. C 73.10, H 9.32, N 3.16; found C 73.15, H 9.08, N 3.21. HRMS (CI): *m*/*z* calcd. for $C_{27}H_{42}NO_4$ $[M + H]^+$ 444.3114; found 444.3077.

(–)-Menthyl (2*SR*,4*R*)-4-[(*tert*-Butoxycarbonyl)amino]-2-methyl-5-phenylpentanoate (14/*epi*-14):[8a] A solution of menthyl ester **13** (599 mg, 1.35 mmol) in methanol (13.5 mL) was stirred under hydrogen with Pd/C (5%, 60 mg) until complete hydrogenation was observed (TLC) and the catalyst was filtered off through a pad of celite. NMR of the crude product showed a 1:3 mixture of *epi*-**14**/**14**. The diastereomers were separated by column chromatography (1. hexane/diethyl ether, 9:1, 2. hexane/EtOAc, 8:2) to give *epi*-**14** (89 mg, 0.200 mmol, 15%, >99% ds), **14** (361 mg, 0.810 mmol, 60%, 98% ds), and a mixed fraction (121 mg, 0.272 mmol, 20%, *epi*-**14**:**14** 2.9:1).

R_f(*epi*-**14**) = 0.42 (hexane/EtOAc, 8:2); m.p. 110–111 °C (*epi*-**14**). $[\alpha]_D^{20}$ = –36.0 (*c* = 1.0, $CHCl_3$, *epi*-**14**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.72 (d, $^3J_{21,20}$ = 6.8 Hz, 3 H, 21-H), 0.84 [dddd, $^2J_{17ax,17eq} \approx {}^3J_{17ax,16ax} \approx {}^3J_{17ax,18(ax)}$ = 12.9, $^3J_{17ax,16eq}$ = 3.6 Hz, 1 H, 17-H_{ax}], 0.86 (d, $^3J_{21',20}$ = 7.0 Hz, 3 H, 21′-H), 0.87 (d, $^3J_{22,18}$ = 6.5 Hz, 3 H, 22-H), 0.90 [ddd, $^2J_{19ax,19eq} \approx {}^3J_{19ax,18ax} \approx {}^3J_{19ax,14(ax)}$ = 11.8 Hz, 1 H, 19-H_{ax}], 1.02 [dddd, $^2J_{16ax,16eq} \approx {}^3J_{16ax,15(ax)} \approx {}^3J_{16ax,17ax}$ = 13.0, $^3J_{16ax,17eq}$ = 3.3 Hz, 1 H, 16-H_{ax}], 1.11 (d, $^3J_{10,2}$ = 7.3 Hz, 3 H, 10-H), 1.29–1.47 [m, 2 H, 15-$H_{(ax)}$, 18-$H_{(ax)}$], 1.38 (s, 9 H, 13-H), 1.49 (ddd, $^2J_{3a,3b}$ = 14.2, $^3J_{3a,2/4}$ = 8.5, $^3J_{3a,2/4}$ = 4.0 Hz, 1 H, 3-H_a), 1.61–1.69 (m, 2 H, 16-H_{eq}, 17-H_{eq}), 1.73 (ddd, $^2J_{3b,3a}$ = 14.2, $^3J_{3b,2/4}$ = 11.0, $^3J_{3b,2/4}$ = 5.5 Hz, 1 H, 3-H_b), 1.80 [qqd, $^3J_{20,21} = {}^3J_{20,21'}$ = 6.9, $^3J_{20,15(ax)}$ = 2.8 Hz, 1 H, 20-H], 1.93 (m, 1 H, 19-H_{eq}), 2.44 (m, 1 H, 2-H), 2.73 (dd, $^2J_{5a,5b}$ = 13.5, $^3J_{5a,4}$ = 6.5 Hz, 1 H, 5-H_a), 2.79 (dd, $^2J_{5b,5a}$ = 13.5, $^3J_{5b,4}$ = 5.0 Hz, 1 H, 5-H_b), 3.87 (br. s, 1 H, 4-H), 4.33 (d, $^3J_{NH,4}$ = 7.8 Hz, 1 H, NH), 4.62 [ddd, $^3J_{14(ax),15(ax)} \approx {}^3J_{14(ax),19ax}$ = 10.9, $^3J_{14(ax),19eq}$ = 4.3 Hz, 1 H, 14-$H_{(ax)}$], 7.14 (d, $^3J_{7,8}$ = 7.0 Hz, 2 H, 7-H), 7.19 (tt, $^3J_{9,8}$ = 7.4, $^4J_{9,7}$ = 1.3 Hz, 1 H, 9-H), 7.26 (dddd, $^3J_{8,7} \approx {}^3J_{8,9}$ = 7.2, $^4J_{8,8'} \approx {}^5J_{8,7'}$ = 1.4 Hz, 2 H, 8-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.2, 16.8, 20.8, 22.0, 23.3, 26.2, 28.4, 31.4, 34.3, 36.9, 37.2, 40.8, 41.2, 47.0, 49.8, 74.1, 79.2, 126.3, 128.3, 129.5, 137.9, 155.4, 176.1 ppm.

R_f(**14**) = 0.38 (hexane/EtOAc, 8:2); m.p. 91–92 °C (**14**). $[\alpha]_D^{20}$ = –17.2 (*c* = 1.0, $CHCl_3$, **14**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.72 (d, $^3J_{21,20}$ = 7.0 Hz, 3 H, 21-H), 0.84 [dddd, $^2J_{17ax,17eq} \approx {}^3J_{17ax,16ax} \approx {}^3J_{17ax,18(ax)}$ = 12.9, $^3J_{17ax,16eq}$ = 3.5 Hz, 1 H, 17-H_{ax}], 0.87 (d, $^3J_{21',20}$ = 7.0 Hz, 3 H, 21′-H), 0.88 (d, $^3J_{22,18}$ = 6.5 Hz, 3 H, 22-H), 0.91 [ddd, $^2J_{19ax,19eq} \approx {}^3J_{19ax,18ax} \approx {}^3J_{19ax,14(ax)}$ = 11.7 Hz, 1 H, 19-H_{ax}], 1.03 [dddd, $^2J_{16ax,16eq} \approx {}^3J_{16ax,15(ax)} \approx {}^3J_{16ax,17ax}$ = 13.0, $^3J_{16ax,17eq}$ = 3.4 Hz, 1 H, 16-H_{ax}], 1.13 (d, $^3J_{10,2}$ = 7.3 Hz, 3 H, 10-H), 1.30–1.54 [m, 3 H, 3-H_a, 15-$H_{(ax)}$, 18-$H_{(ax)}$], 1.37 (s, 9 H, 13-H), 1.61–1.69 (m, 2 H, 16-H_{eq}, 17-H_{eq}), 1.78–1.91 (m, 2 H, 3-H_b, 20-H), 1.97 (d, $^2J_{19eq,19ax}$ = 11.5 Hz, 1 H, 19-H_{eq}), 2.54 (m, 1 H, 2-H), 2.75 (dd, $^2J_{5a,5b}$ = 13.2, $^3J_{5a,4}$ = 6.1 Hz, 1 H, 5-H_a), 2.79 (m, 1 H, 5-H_b), 3.85 (br. s, 1 H, 4-H), 4.32 (br. s, 1 H, NH), 4.65 [ddd, $^3J_{14(ax),15(ax)} \approx {}^3J_{14(ax),19ax}$ = 10.9, $^3J_{14(ax),19eq}$ = 4.3 Hz, 1 H, 14-$H_{(ax)}$], 7.15 (d, $^3J_{7,8}$ = 7.3 Hz, 2 H, 7-H), 7.19 (tt, $^3J_{9,8}$ = 7.3, $^4J_{9,7}$ = 1.2 Hz, 1 H, 9-H), 7.26 (dddd, $^3J_{8,7} \approx {}^3J_{8,9}$ = 7.3, $^4J_{8,8'} \approx {}^5J_{8,7'}$ = 1.3 Hz, 2 H, 8-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.1, 17.7, 20.8, 22.0, 23.3, 26.2, 28.4, 31.4, 34.3, 36.7, 37.6, 40.8, 41.2, 47.1, 49.9, 74.1, 79.0, 126.3, 128.3, 129.5, 137.9, 155.1, 175.7 ppm.

HPLC: Lichrosorb Si-60 5µ, hexane/EtOAc, 95:5, 2 mL min^{-1}, t_R(*epi*-**14**) = 7.83 min, t_R(**14**) = 10.17 min. HRMS (CI): *m*/*z* calcd. for $C_{27}H_{44}NO_4$ $[M + H]^+$ 446.3270; found 446.3280. $C_{27}H_{43}NO_4$ (445.64): calcd. C 72.77, H 9.73, N 3.14; found C 72.42, H 9.49, N 3.16.

Methyl (2*S*,4*R*)-4-Amino-2-methyl-5-phenylpentanoate, Hydrochloride (15):[21] As described in the literature,[8a] the menthyl ester **14** (334 mg, 0.750 mmol) was deprotected and converted into the methyl ester **15** (138 mg, 0.535 mmol, 71%). $[\alpha]_D^{20}$ = +11.2 (*c* = 1.0, MeOH); m.p. 134–137 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.13 (d, $^3J_{10,2}$ = 7.0 Hz, 3 H, 10-H), 1.78 (ddd, $^2J_{3a,3b}$ = 13.8, $^3J_{3a,2/4}$ = 8.9, $^3J_{3a,2/4}$ = 3.8 Hz, 1 H, 3-H_a), 1.97 (ddd, $^2J_{3b,3a}$ = 13.8, $^3J_{3b,2/4}$ = 10.7, $^3J_{3b,2/4}$ = 3.0 Hz, 1 H, 3-H_b), 2.90 (dd, $^2J_{5a,5b}$ = 13.6, $^3J_{5a,4}$ = 8.9 Hz, 1 H, 5-H_a), 2.92 (m, 1 H, 2-H), 3.27 (dd, $^2J_{5b,5a}$ = 13.6, $^3J_{5b,4}$ = 5.3 Hz, 1 H, 5-H_b), 3.58 (s, 3 H, 11-H), 3.60 (br. s, 1 H, 4-H), 7.20–7.26 (m, 3 H, 7-H, 9-H), 7.30 (dd, $^3J_{8,7} \approx {}^3J_{8,9}$ = 7.2 Hz, 2 H, 8-H), 8.52 (br. s, 3 H, NH_3^+) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.7, 35.9, 36.0, 39.7, 51.9, 52.2, 127.3, 128.9, 129.3, 135.4, 175.7 ppm.

Methyl (4*R*)-4-Amino-5-phenylpentanoate, Hydrochloride (16): Boc-L-phenylalanine methyl ester (4.27 g, 15.3 mmol) was reduced to the aldehyde with DibalH (1 M solution in hexane, 33 mL, 33 mmol) by the General Procedure for in situ DibalH reduction/Wittig reaction and elongated with methyl (triphenyl-λ^5-phosphanyliden)acetate (10.4 g, 31.1 mmol). The Wittig product was obtained after flash chromatography (hexane/EtOAc, 8:2) as white solid (2.59 g, 8.47 mmol, 55%). A solution of the Wittig product (1.37 g, 4.49 mmol) was stirred under hydrogen with Pd/C (10%, 68 mg) until complete hydrogenation was observed (TLC) and the catalyst was filtered off through a pad of celite. Purification by flash chromatography (hexane/EtOAc, 8:2) afforded the saturated intermediate as a white solid (1.33 g, 4.34 mmol, 96%). A solution of HCl in dioxane (4 M, 6.25 mL, 25 mmol) was added at 0 °C to the hydrogenation product (768 mg, 2.5 mmol). The solvent was evaporated in vacuo after complete deprotection (TLC monitoring) and the hydrochloride salt was dried in high vacuum to give the salt **16** (599 mg, 2.46 mmol, 98%) as a white solid; m.p. 117–119 °C. $[\alpha]_D^{20}$ = –7.4 (*c* = 1.0, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.02 (td, $^3J_{3,2} \approx {}^3J_{3,4}$ = 7.0 Hz, 2 H, 3-H), 2.54 (dt, $^2J_{2a,2b}$ = 16.8, $^3J_{2a,3}$ = 7.5 Hz, 1 H, 2-H_a), 2.62 (dt, $^2J_{2b,2a}$ = 16.8, $^3J_{2b,3}$ = 7.5 Hz, 1 H, 2-H_b), 2.93 (dd, $^2J_{5a,5b}$ = 13.7, $^3J_{5a,4}$ = 8.9 Hz, 1 H, 5-H_a), 3.27 (dd, $^2J_{5b,5a}$ = 13.7, $^3J_{5b,4}$ = 5.2 Hz, 1 H, 5-H_b), 3.55 (s, 3 H, 10-H), 3.60 (br. s, 1 H, 4-H), 7.20–7.26 (m, 3 H, 7-H, 9-H), 7.29 (dd, $^3J_{8,7} \approx {}^3J_{8,9}$ = 7.3 Hz, 2 H, 8-H), 8.51 (br. s, 3 H, NH_3^+) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 27.0, 30.1, 39.3, 51.8, 53.2, 127.3, 128.9, 129.4, 135.5, 127.9 ppm. HRMS (EI): *m*/*z* calcd. for $C_{11}H_{14}NO$ $[M - CH_3O]^+$ 176.1075; found 176.1059.

General Procedure for Mixed-Anhydride Peptide Coupling: Isobutyl chloroformate (1.1 equiv.) was added dropwise at –20 °C to a solution of carboxylic acid (1.0 equiv.) and *N*-methylmorpholine (1.1 equiv.) in dry THF (10 mL mmol^{-1}). After 10 min, either a solution of free amine (1.0 equiv.) in dry THF (2 mL mmol^{-1}) was added dropwise or the amine was added as the solid hydrochloride (1.0 equiv.) after addition of further *N*-methylmorpholine (1.1 equiv.). The mixture was allowed to warm to room temperature overnight. The white precipitate (hydrochloride salt of *N*-methylmorpholine) was filtered off and the filtrate was concentrated in vacuo. The residue was dissolved in EtOAc, and washed with water, which was extracted afterwards twice with EtOAc. The combined organic layers were washed with saturated $NaHCO_3$ and dried with Na_2SO_4.

Methyl (4*R*)-4-[((*R*)-2-{3-[(*N*-Benzyloxycarbonyl-(*S*)-isoleucyl)methylamino]-4-methylpentyl}thiazole-4-carbonyl)amino]-5-phenylpentanoate (17): The free acid was obtained as a pale yellow foam (139 mg, 0.284 mmol, 95%) by saponification of the dipeptide **7** (155 mg, 0.299 mmol) with NaOH (1 M, 450 µL, 0.45 mmol). This acid (49 mg, 100 µmol) was coupled with the hydrochloride **16** (37 mg, 152 µmol) by the General Procedure for mixed anhydride peptide coupling, with the aid of isobutyl chloroformate (14 µL, 108 µmol) and *N*-methylmorpholine (2 × 12 µL, 218 µmol). Purification by flash chromatography (hexane/diethyl ether, 3:7, 2:8)

yielded the tripeptide **17** (57 mg, 84.0 μmol, 84%) as a colorless resin. R_f = 0.24 (Hex/EtOAc, 1:1). $[\alpha]_D^{20}$ = –10.9 (*c* = 1.0, $CHCl_3$). Major rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.76 (d, $^3J_{18,17}$ = 6.5 Hz, 3 H, 18-H), 0.85 (t, $^3J_{24,23}$ = 7.4 Hz, 3 H, 24-H), 0.95 (d, $^3J_{25,22}$ = 6.5 Hz, 3 H, 25-H), 0.97 (d, $^3J_{18',17}$ = 6.3 Hz, 3 H, 18′-H), 1.10 (ddq, $^2J_{23a,23b}$ = 13.6, $^3J_{23a,22}$ = 9.8, $^3J_{23a,24}$ = 7.3 Hz, 1 H, 23-H_a), 1.57 (dqd, $^2J_{23b,23a}$ = 13.6, $^3J_{23b,24}$ = 7.6, $^3J_{23b,22}$ = 2.9 Hz, 1 H, 23-H_b), 1.65–1.90 (m, 4 H, 3-H_a, 15-H_a, 17-H, 22-H), 1.95 (m, 1 H, 3-H_b), 2.35 (ddd, $^2J_{2a,2b}$ = 16.3, $^3J_{2a,3a/b}$ = 9.3, $^3J_{2a,3a/b}$ = 6.5 Hz, 1 H, 2-H_a), 2.43 (ddd, $^2J_{2b,2a}$ = 16.3, $^3J_{2b,3a/b}$ = 9.8, $^3J_{2b,3a/b}$ = 6.0 Hz, 1 H, 2-H_b), 2.79 (t, $^3J_{14,15}$ = 8.2 Hz, 2 H, 14-H), 2.82 (dd, $^2J_{5a,5b}$ = 13.3, $^3J_{5a,4}$ = 7.2 Hz, 1 H, 5-H_a), 2.96 (s, 3 H, 19-H), 2.97 (dd, $^2J_{5b,5a}$ = 13.3, $^3J_{5b,4}$ = 5.8 Hz, 1 H, 5-H_b), 3.55 (s, 3 H, 32-H), 4.28–4.43 (m, 2 H, 4-H, 16-H), 4.54 (dd, $^3J_{21,NH}$ = 9.4, $^3J_{21,22}$ = 7.0 Hz, 1 H, 21-H), 5.06 (d, $^2J_{27a,27b}$ = 12.5 Hz, 1 H, 27-H_a), 5.10 (d, $^2J_{27b,27a}$ = 12.5 Hz, 1 H, 27-H_b), 5.47 (d, $^3J_{NH,21}$ = 9.4 Hz, 1 H, NH_{Ile}), 7.15–7.34 (m, 10 H, arom. H), 7.37 (d, $^3J_{NH,4}$ = 9.0 Hz, 1 H, $NH_{\text{“Phe”}}$), 7.89 (s, 1 H, 12-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2, 15.9, 19.5, 20.1, 23.9, 29.1, 29.3, 29.6, 29.9*, 30.2, 31.0, 37.6, 41.5, 49.8, 51.5, 55.8, 58.6*, 66.8, 122.4, 126.5, 127.8, 128.0, 128.4, 128.4, 129.4, 136.4, 137.7, 149.7, 156.5, 160.9, 169.6, 173.3, 173.9 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86 (t, $^3J_{24,23}$ = 7.3 Hz, 3 H, 24-H), 0.92, 0.93 (2 × d, $^3J_{18,17}$ = $^3J_{25,22}$ = 6.8 Hz, 6 H, 18-H, 25-H), 1.06 (d, $^3J_{18',17}$ = 6.5 Hz, 3 H, 18′-H), 2.73 (s, 3 H, 19-H), 3.56 (s, 3 H, 32-H), 4.64 (dd, $^3J_{21,NH}$ = 9.8, $^3J_{21,22}$ = 6.8 Hz, 1 H, 21-H), 5.04 (d, $^2J_{27a,27b}$ = 12.3 Hz, 1 H, 27-H_a), 5.54 (d, $^3J_{NH,21}$ = 9.8 Hz, 1 H, NH_{Ile}), 7.88 (s, 1 H, 12-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.3, 16.2, 20.4, 23.5, 27.4, 29.2, 31.0, 37.9, 50.0, 55.3, 62.7, 66.9, 122.7, 137.8, 169.8, 172.7 ppm. HRMS (CI): *m*/*z* calcd. for $C_{37}H_{51}N_4O_6S$ [M – CH_2]$^+$ 679.3529; found 679.3581. $C_{38}H_{53}N_4O_6S$ (693.92): calcd. C 65.77, H 7.70, N 8.07; found C 65.39, H 7.35, N 8.35.

Methyl (4*R*)-4-[((*R*)-2-{3-[(*N*,*N*-Dimethylglycyl-(*S*)-isoleucyl)methylamino]-4-methylpentyl}thiazole-4-carbonyl)amino]-5-phenylpentanoate (18): Pentafluorophenol (202 mg, 1.1 mmol) and dicyclohexyl carbodiimide (227 mg, 1.10 mmol) were added to a solution of *N*-methylsarcosine (103 mg, 0.999 mmol) in EtOAc, (2.5 mL). After the system had been stirred overnight, precipitated urea was filtered off through a pad of celite and the solvent was evaporated in vacuo.[7a] The tripeptide **17** (128 mg, 0.189 mmol) was *N*-deprotected with HBr in acetic acid (33 wt.-%, 330 μL, 1.91 mmol) at 0 °C and the resulting hydrobromide was deprotonated with saturated $NaHCO_3$ to afford the free amine, which was treated with *N*-methylsarcosine pentafluorophenol ester (195 mg, 0.724 mmol) in dichloromethane (1.9 mL) overnight. The tetrapeptide **18** (66 mg, 0.105 mmol, 55%) was obtained by flash chromatography (hexane/EtOAc, 1:1, 0:100; CH_2Cl_2/MeOH, 98:2, 95:5) as a pale yellow oil. R_f = 0.38 (CH_2Cl_2/MeOH, 9:1). $[\alpha]_D^{20}$ = –25.7 (*c* = 1.0, $CHCl_3$). Major rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.74 (d, $^3J_{18,17}$ = 6.5 Hz, 3 H, 18-H), 0.84 (t, $^3J_{24,23}$ = 7.3 Hz, 3 H, 24-H), 0.94 (2 × d, $^3J_{18',17}$ = $^3J_{25,22}$ = 6.8 Hz, 6 H, 18′-H, 25-H), 1.10 (ddq, $^2J_{23a,23b}$ = 13.8, $^3J_{23a,22}$ = 9.8 Hz, $^3J_{23a,24}$ = 7.3 Hz, 1 H, 23-H_a), 1.56 (dqd, $^2J_{23b,23a}$ = 13.8, $^3J_{23b,24}$ = 7.2, $^3J_{23b,22}$ = 2.8 Hz, 1 H, 23-H_b), 1.69 (m, 1 H, 17-H), 1.74–1.89 (m, 3 H, 3-H_a, 15-H_a, 22-H), 1.94 (m, 1 H, 3-H_b), 2.09 (dtd, $^2J_{15b,15a}$ = 14.8, $^3J_{15b,14}$ = 8.5, $^3J_{15,16}$ = 3.4 Hz, 1 H, 15-H_b), 2.24 (s, 6 H, 28-H), 2.34 (ddd, $^2J_{2a,2b}$ = 16.1, $^3J_{2a,3a/b}$ = 8.5, $^3J_{2a,3a/b}$ = 6.7 Hz, 1 H, 2-H_a), 2.40 (ddd, $^2J_{2b,2a}$ = 16.1, $^3J_{2b,3a/b}$ = 9.1, $^3J_{2b,3a/b}$ = 6.4 Hz, 1 H, 2-H_b), 2.75–3.02 (m, 6 H, 5-H, 14-H, 27-H), 2.97 (s, 3 H, 19-H), 3.55 (s, 3 H, 29-H), 4.28–4.43 (m, 2 H, 4-H, 16-H), 4.81 (dd, $^3J_{21,NH}$ = 9.7, $^3J_{21,22}$ = 7.5 Hz, 1 H, 21-H), 7.13–7.27 (m, 5 H, arom. H), 7.36 (d, $^3J_{NH,4}$ = 9.3 Hz, 1 H, $NH_{\text{“Phe”}}$), 7.59 (d, $^3J_{NH,21}$ = 9.7 Hz, 1 H, NH_{Ile}), 7.87 (s, 1 H, 12-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.1, 15.9, 19.5, 20.0, 24.2, 29.1, 29.2, 29.6*, 30.0, 30.2, 30.9, 37.3, 41.4, 45.9, 49.8, 51.5, 53.1, 58.5*, 63.0, 122.3, 126.4, 128.4, 129.4, 137.6, 149.7, 160.8, 169.6, 170.4, 173.0, 173.7 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.90 (d, $^3J_{18,17/25,22}$ = 6.8 Hz, 3 H, 18′-H/25-H), 1.06 (d, $^3J_{18',17}$ = 6.5 Hz, 3 H, 18′-H), 2.65 (ddd, $^3J_{14a,14b}$ = 15.8, $^3J_{14a,15a/b}$ = 10.9, $^3J_{14,15a/b}$ = 4.6 Hz, 1 H, 14-H_a), 2.76 (s, 3 H, 19-H), 3.64 (ddd, $^3J_{16,15a} \approx {}^3J_{16,17}$ = 10.2, $^3J_{16,15b}$ = 3.0 Hz, 1 H, 16-H), 4.92 (dd, $^3J_{21,NH}$ = 9.8, $^3J_{21,22}$ = 6.8 Hz, 1 H, 21-H), 7.30 (d, $^3J_{NH,4}$ = 9.5 Hz, 1 H, $NH_{\text{“Phe”}}$), 7.66 (d, $^3J_{NH,21}$ = 9.8 Hz, 1 H, NH_{Ile}), 7.86 (s, 1 H, 12-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2, 16.3, 20.3, 20.4, 23.9, 27.3, 31.3, 37.9, 46.0, 49.9, 52.5, 62.6, 63.1, 122.6, 149.6, 169.8, 170.1, 172.6, 173.7 ppm. HRMS (CI): *m*/*z* calcd. for $C_{33}H_{52}N_5O_5S$ [M + H]$^+$ 630.3689; found 630.3687.

(4*R*)-4-[((*R*)-2-{3-[(*N*,*N*-Dimethylglycyl-(*S*)-isoleucyl)methylamino]-4-methylpentyl}thiazole-4-carbonyl)amino]-5-phenylpentanoic Acid, Trifluoroacetic Acid Salt (19): A mixture of the tetrapeptide **18** (52 mg, 82.6 μmol) and NaOH (1 M, 165 μL, 165 μmol) in dioxane (0.8 mL) was stirred at 0 °C until complete saponification. The solvent was evaporated in vacuo, and the residue was dissolved in water, acidified to pH 2 with trifluoroacetic acid, and extracted with EtOAc. The combined organic layers were dried with Na_2SO_4 and the solvent was evaporated in vacuo. Purification by flash chromatography (CH_2Cl_2/MeOH, 9:1) gave **19** as a white solid in quantitative yield. R_f = 0.09 (CH_2Cl_2/MeOH, 9:1); m.p. 55 °C. $[\alpha]_D^{20}$ = –26.1 (*c* = 1.0, MeOH). Major rotamer: ^{1}H NMR (400 MHz, MeOD): δ = 0.78 (d, $^3J_{18,17}$ = 6.5 Hz, 3 H, 18-H), 0.92 (t, $^3J_{24,23}$ = 7.4 Hz, 3 H, 24-H), 0.97 (d, $^3J_{18',17}$ = 6.6 Hz, 3 H, 18′-H), 1.02 (d, $^3J_{25,22}$ = 6.8 Hz, 3 H, 25-H), 1.21 (ddq, $^2J_{23a,23b}$ = 13.8, $^3J_{23a,22}$ = 9.6, $^3J_{23a,24}$ = 7.3 Hz, 1 H, 23-H_a), 1.64 (dqd, $^2J_{23b,23a}$ = 13.8, $^3J_{23b,24}$ = 7.5, $^3J_{23b,22}$ = 2.8 Hz, 1 H, 23-H_b), 1.78 (m, 1 H, 17-H), 1.84–2.06 (m, 4 H, 3-H, 15-H_a, 17-H, 22-H), 2.16 (dtd, $^2J_{15b,15a}$ = 14.5, $^3J_{15b,14}$ = 8.1, $^3J_{15b,16}$ = 3.3 Hz, 1 H, 15-H_b), 2.32 (dt, $^2J_{2a,2b}$ = 15.9, $^3J_{2a,3}$ = 7.6 Hz, 1 H, 2-H_a), 2.41 (ddd, $^2J_{2b,2a}$ = 15.9, $^3J_{2b,3a/b}$ = 8.7, $^3J_{2b,3a/b}$ = 6.2 Hz, 1 H, 2-H_b), 2.72 (s, 6 H, 28-H), 2.80–3.03 (m, 4 H, 5-H, 14-H), 3.08 (s, 3 H, 19-H), 3.68 (d, $^2J_{27a,27b}$ = 15.6 Hz, 1 H, 27-H_a), 3.73 (d, $^2J_{27b,27a}$ = 15.6 Hz, 1 H, 27-H_b), 4.26–4.39 (m, 2 H, 4-H, 16-H), 4.79 (d, $^3J_{21,22}$ = 7.8 Hz, 1 H, 21-H), 7.16 (m, 1 H, 9-H), 7.20–7.26 (m, 4 H, 7-H, 8-H), 7.97 (s, 1 H, 12-H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 11.4, 16.1, 20.2, 20.5, 25.4, 30.3, 30.9*, 30.8, 31.0, 31.3, 32.9, 37.9, 42.1, 44.8, 52.0, 55.7, 59.9*, 60.4, 118.2 [q, $^1J_{C,F}$ = 290.9 Hz], 124.2, 127.4, 129.3, 130.4, 139.6, 150.4, 163.1 (q, $^2J_{C,F}$ = 35.6 Hz), 163.2, 167.6, 171.8, 174.5, 178.9 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, MeOD): δ = 1.11 (d, $^3J_{18',17}$ = 6.5 Hz, 3 H, 18′-H), 2.68 (s, 6 H, 28-H), 2.77 (s, 1 H, 19-H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 11.6, 16.5, 21.0, 24.7, 32.5, 38.4, 41.9, 45.0, 54.8, 124.4 ppm. HRMS (CI): *m*/*z* calcd. for $C_{32}H_{48}N_5O_4S$ [M – OH]$^+$ 598.3427; found 598.3447.

Ethyl (*R*)-2-{3-[(*N*-Benzyloxycarbonyl-(*R*)-pipecolyl-(*S*)-isoleucyl)-methylamino]-4-methylpentyl}thiazole-4-carboxylate (20): The dipeptide **7** (779 mg, 1.50 mmol) was *N*-deprotected with HBr in acetic acid (33 wt.-%, 2.59 mL, 15 mmol) at 0 °C and the resulting hydrobromide was deprotonated with saturated $NaHCO_3$ to afford the free amine, which was coupled with (*R*)-Z-pipecolic acid (434 mg, 1.65 mmol, >98% *ee*) with the aid of isobutyl chloroformate (214 μL, 1.65 mmol) and *N*-methylmorpholine (198 μL, 1.80 mmol) by the General Procedure for mixed anhydride peptide coupling to afford the tripeptide **20** (720 mg, 1.15 mmol, 76%, >99% *de*) as a colorless oil after flash chromatography (hexane/EtOAc, 6:4, 1:1, 3:7). R_f = 0.18 (hexane/EtOAc, 1:1). HPLC: Lichrosorb Si-60 5 μ, hexane/EtOAc, 8:2–6:4, 2 mL min^{-1}, t_R[(*R*,*S*,*R*)-

20] = 26.04 min, t_R[(*S*,*S*,*R*)-**20**] = 28.22 min. $[\alpha]_D^{20}$ = +3.8 (*c* = 1.0, $CHCl_3$). Major rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.73 (d, $^3J_{9,8}$ = 6.8 Hz, 3 H, 9-H), 0.81 (m, 3 H, 15-H), 0.90 (m, 3 H, 16-H), 0.93 (d, $^3J_{9',8}$ = 6.8 Hz, 3 H, 9′-H), 1.01 (m, 1 H, 14-H_a), 1.25–1.41 (m, 2 H, 20-H_{ax}, 21-H_{ax}),1.36 (t, $^3J_{30,29}$ = 7.2 Hz, 3 H, 30-H), 1.41–1.63 (m, 4 H, 14-H_b, 19-H_{ax}, 20-H_{eq}, 21-H_{eq}), 1.67 (m, 1 H, 8-H), 1.73 (m, 1 H, 13-H), 1.87 (m, 1 H, 6-H_a), 2.14 (m, 1 H, 6-H_b), 2.27 (d, $^2J_{19eq,19ax}$ = 12.8 Hz, 1 H, 19-H_{eq}), 2.77–2.86 (m, 2 H, 5-H_a, 22-H_{ax}), 2.89 (ddd, $^2J_{5b,5a}$ = 15.1, $^3J_{5b,6a/b}$ = 6.2, $^3J_{5b,6a/b}$ = 3.3 Hz, 1 H, 5-H_b), 2.96 (s, 3 H, 10-H), 4.07 (br. s, 1 H, 22-H_{eq}), 4.32 (m, 1 H, 7-H), 4.37 (q, $^3J_{29,30}$ = 7.2 Hz, 2 H, 29-H), 4.78 (dd, $^3J_{12,NH} \approx {}^3J_{12,13}$ = 8.3 Hz, 1 H, 12-H), 4.87 (br. s, 1 H, 18-H), 5.11 (d, $^2J_{24a,24b}$ = 11.6 Hz, 1 H, 24-H_a), 5.15 (d, $^2J_{24b,24a}$ = 12.3 Hz, 1 H, 24-H_b), 6.55 (br. s, 1 H, NH_{Ile}), 7.22–7.40 (m, 5 H, arom. H), 8.00 (s, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.0, 14.3, 15.9, 19.5, 19.9, 20.4, 24.1, 24.7*, 25.9*, 29.3, 29.7, 30.1, 30.6, 37.1, 42.2*, 53.6, 55.2*, 58.9, 61.3, 67.6, 126.9, 127.8, 128.0, 128.5, 136.3, 146.9, 156.4, 161.3, 170.3, 170.8, 173.2 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.03 (d, $^3J_{9',8}$ = 6.3 Hz, 3 H, 9′-H), 2.73 (s, 3 H, 10-H), 3.61 (m, 1 H, 7-H), 4.17 (br. s, 1 H, 22-H_{eq}), 4.92 (dd, $^3J_{12,NH}$ = 9.6, $^3J_{12,13}$ = 6.0 Hz, 1 H, 12-H), 5.06 (d, $^2J_{24a,24b}$ = 12.6 Hz, 1 H, 24-H_a), 7.95 (s, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2, 15.2, 16.2, 20.3, 20.5, 23.4, 27.4, 29.9, 31.3, 53.0, 61.3, 62.7, 67.5, 126.9, 146.8, 171.0 ppm. HRMS (CI): *m*/*z* calcd. for $C_{33}H_{49}N_4O_6S$ $[M + H]^+$ 629.3373; found 629.3327. $C_{33}H_{48}N_4O_6S$ (628.83): calcd. C 63.03, H 7.69, N 8.91; found C 63.07, H 7.36, N 9.16.

(*R*)-2-{3-[(*N*-Benzyloxycarbonyl-(*R*)-pipecolyl-(*S*)-isoleucyl)methylamino]-4-methylpentyl}thiazole-4-carboxylic Acid (21): A mixture of ethyl ester **20** (554 mg, 0.881 mmol) and NaOH (1 M, 1.32 mL, 1.32 mmol) in dioxane (9 mL) was stirred at 0 °C until complete saponification. The solvent was evaporated in vacuo, and the residue was dissolved in water, acidified to pH 2 with HCl (1 M), and extracted twice with EtOAc. The combined organic layers were dried with Na_2SO_4 and the solvent was evaporated in vacuo to give the free acid **21** (518 mg, 0.862 mmol, 98%) as a colorless glass-like solid; m.p. 78–80 °C. $[\alpha]_D^{20}$ = +6.5 (*c* = 1.0, $CHCl_3$). Major rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.74 (d, $^3J_{9,8}$ = 6.5 Hz, 3 H, 9-H), 0.81 (m, 3 H, 15-H), 0.88–0.98 (m, 6 H, 9′-H, 16-H), 1.06 (m, 1 H, 14-H_a), 1.24–1.74 (m, 7 H, 8-H, 14-H_b, 19-H_{ax}, 20-H, 21-H), 1.78–1.95 (m, 2 H, 6-H_a, 13-H), 2.13 (m, 1 H, 6-H_b), 2.29 (d, $^2J_{19eq,19ax}$ = 13.3 Hz, 1 H, 19-H_{eq}), 2.83 (m, 1 H, 22-H_{ax}), 2.87 (t, $^3J_{5,6}$ = 7.8 Hz, 2 H, 5-H), 3.02 (s, 3 H, 10-H), 4.07 (br. s, 1 H, 22-H_{eq}), 4.37 (m, 1 H, 7-H), 4.81 (dd, $^3J_{12,NH} \approx {}^3J_{12,13}$ = 8.7 Hz, 1 H, 12-H), 4.90 (br. s, 1 H, 18-H), 5.12 (d, $^2J_{24a,24b}$ = 12.5 Hz, 1 H, 24-H_a), 5.17 (d, $^2J_{24b,24a}$ = 12.5 Hz, 1 H, 24-H_b), 6.85 (br. s, 1 H, NH), 7.23–7.37 (m, 5 H, arom. H), 8.10 (s, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.8, 15.8, 19.5, 19.9, 20.4, 24.3, 24.8*, 26.0*, 29.6, 30.2, 30.4, 36.7*, 42.1*, 53.8, 55.1*, 59.1, 67.6, 127.6, 127.8, 128.0, 128.4, 136.4, 146.5, 163.2, 170.5, 170.8, 174.0 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.03 (d, $^3J_{9',8}$ = 6.5 Hz, 3 H, 9′-H), 2.73 (s, 3 H, 10-H), 3.64 (m, 1 H, 7-H), 4.14 (br. s, 1 H, 22-H_{eq}), 6.95 (br. s, 1 H, NH), 8.06 (s, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2, 16.1, 20.2, 20.5, 23.6, 27.6, 53.1, 62.8 ppm. $C_{31}H_{44}N_4O_6S$ (600.78): calcd. C 61.98, H 7.38, N 9.33; found C 61.92, H 7.47, N 9.39. HRMS (CI): *m*/*z* calcd. for $C_{30}H_{45}N_4O_4S$ $[M - CO_2 + H]^+$ 557.3162; found 557.3186.

Methyl (4*R*)-4-[((*R*)-2-{3-[(*N*-Benzyloxycarbonyl-(*R*)-pipecolyl-(*S*)-isoleucyl)methylamino]-4-methylpentyl}thiazole-4-carbonyl)amino]-5-phenylpentanoate (22): The free acid **21** (60 mg, 100 μmol) and the hydrochloride **16** (27 mg, 111 μmol) were coupled by the General Procedure for mixed anhydride peptide coupling with the aid of isobutyl chloroformate (14 μL, 108 μmol) and *N*-methylmorpholine (2 × 12 μL, 218 μmol) to give the tetrapeptide **22** (65 mg, 82.2 μmol, 82%) as a colorless resin after column chromatography (hexane/EtOAc, 1:1, 3:7). R_f = 0.14 (Hex/EtOAc, 1:1). $[\alpha]_D^{20}$ = +4.8 (*c* = 1.0, $CHCl_3$). Major rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.76 (d, $^3J_{18,17}$ = 6.3 Hz, 3 H, 18-H), 0.83 (m, 3 H, 24-H), 0.91 (d, $^3J_{25,22}$ = 6.3 Hz, 3 H, 25-H), 0.96 (d, $^3J_{18',17}$ = 6.5 Hz, 3 H, 18′-H), 1.04 (m, 1 H, 23-H_a), 1.18–1.89 (m, 10 H, 3-H_a, 15-H_a, 17-H, 22-H, 23-H_b, 28-H_{ax}, 29-H, 30-H), 1.96 (m, 1 H, 3-H_b), 2.12 (m, 1 H, 15-H_b), 2.29 (d, $^2J_{28eq,28ax}$ = 13.3 Hz, 1 H, 28-H_{ax}), 2.35 (dt, $^2J_{2a,2b}$ = 16.7, $^3J_{2a,3}$ = 6.8 Hz, 1 H, 2-H_a), 2.42 (dt, $^2J_{2b,2a}$ = 16.7, $^3J_{2b,3}$ = 7.1 Hz, 1 H, 2-H_b), 2.73–2.91 (m, 3 H, 14-H, 31-H_{ax}), 2.82 (dd, $^2J_{5a,5b}$ = 13.6, $^3J_{5a,4}$ = 7.3 Hz, 1 H, 5-H_a), 2.96 (dd, $^2J_{5b,5a}$ = 13.6, $^3J_{5b,4}$ = 6.0 Hz, 1 H, 5-H_b), 3.00 (s, 3 H, 19-H), 3.57 (s, 3 H, 38-H), 4.09 (br. s, 1 H, 31-H_{eq}), 4.29–4.43 (m, 2 H, 4-H, 16-H), 4.81 (dd, $^3J_{21,NH} \approx {}^3J_{21,22}$ = 8.4 Hz, 1 H, 21-H), 4.90 (br. s, 1 H, 27-H), 5.12 (d, $^2J_{33a,33b}$ = 12.0 Hz, 1 H, 33-H_a), 5.17 (d, $^2J_{33b,33a}$ = 12.0 Hz, 1 H, 33-H_b), 6.59 (br. s, 1 H, NH_{Ile}), 7.16–7.39 (m, 11 H, arom. H, $NH_{\text{“Phe”}}$), 7.89 (s, 1 H, 12-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.0, 15.8, 19.6, 20.0, 20.4, 24.2, 24.7*, 25.9*, 29.2, 29.3, 29.3*, 30.1, 30.2, 31.0, 37.2, 41.5, 42.1*, 49.8, 51.5, 53.6, 55.1*, 58.7, 67.6, 122.5, 126.5, 127.8, 128.1, 128.4, 128.5, 129.4, 136.3, 137.6, 149.7, 156.4, 160.8, 169.7, 170.5, 173.2, 173.8 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.06 (d, $^3J_{18',17}$ = 6.5 Hz, 3 H, 18′-H), 2.77 (s, 3 H, 19-H), 4.19 (br. s, 1 H, 31-H_{eq}), 4.99 (dd, $^3J_{21,NH}$ = 9.3, $^3J_{21,22}$ = 6.5 Hz, 1 H, 21-H), 5.07 (d, $^2J_{33a,33b}$ = 12.3 Hz, 1 H, 33-H_a), 7.87 (s, 1 H, 12-H) ppm. HRMS (CI): *m*/*z* calcd. for $C_{36}H_{52}N_5O_7S$ $[M - C_7H_7]^+$ 698.3587; found 698.3591.

(4*R*)-4-[((*R*)-2-{3-[Methyl-(*N*-methyl-(*R*)-pipecolyl-(*S*)-isoleucyl)amino]-4-methylpentyl}thiazole-4-carbonyl)amino]-5-phenylpentanoic Acid, Trifluoroacetic Acid Salt (23): The tetrapeptide **22** (64 mg, 75.3 μmol) was *N*-deprotected with HBr in acetic acid (33 wt.-%, 110 μL, 637 μmol) at 0 °C and the resulting hydrobromide was deprotonated with saturated $NaHCO_3$ to afford the free amine. This *N*-deprotected peptide was dissolved in MeOH (0.75 mL), and paraformaldehyde (7.0 mg, 77.7 μmol) was added. After the system had been stirred for 3 h at room temperature, sodium cyanoborohydride (5.2 mg, 82.7 μmol) was added. When the reaction was finished (TLC monitoring), methanol was removed in vacuo and the residue was dissolved in dichloromethane and washed with saturated $NaHCO_3$. The aqueous layer was extracted with dichloromethane and the combined organic layers were dried with Na_2SO_4. The *N*-methylated tetrapeptide (37 mg, 55.2 μmol, 73%) was obtained after flash chromatography (CH_2Cl_2/MeOH, 95:5, 9:1) as a yellow oil. A mixture of the *N*-methylated tetrapeptide (37 mg, 55.2 μmol) and NaOH (1 M, 110 μL, 110 μmol) in dioxane (0.55 mL) was stirred at 0 °C until complete saponification. The solvent was evaporated in vacuo, and the residue was dissolved in water, acidified to pH 2 with trifluoroacetic acid, and extracted twice with EtOAc. The combined organic layers were dried with Na_2SO_4 and the solvent was evaporated in vacuo. Purification by flash chromatography (CH_2Cl_2/MeOH, 9:1) gave **23** as white solid in quantitative yield. R_f = 0.11 (CH_2Cl_2/MeOH, 9:1); m.p. 174–178 °C. $[\alpha]_D^{20}$ = –12.7 (*c* = 1.0, MeOH). Major rotamer: ^{1}H NMR (400 MHz, MeOD): δ = 0.78 (d, $^3J_{18,17}$ = 6.5 Hz, 3 H, 18-H), 0.91 (t, $^3J_{24,23}$ = 7.5 Hz, 3 H, 24-H), 0.97 (d, $^3J_{18',17}$ = 6.5 Hz, 3 H, 18′-H), 1.01 (d, $^3J_{25,22}$ = 6.8 Hz, 3 H, 25-H), 1.23 (ddq, $^2J_{23a,23b}$ = 13.7, $^3J_{23a,22}$ = 9.0, $^3J_{23a,24}$ = 7.3 Hz, 1 H, 23-H_a), 1.48 (ddddd, $^2J_{29ax,29eq} \approx {}^3J_{29ax,28ax} \approx {}^3J_{29ax,30ax}$ = 12.7, $^3J_{29ax,28eq} \approx {}^3J_{29ax,30eq}$ = 3.4 Hz, 1 H, 29-H_{ax}), 1.56–2.06 (m, 11 H, 3-H, 15-H_a, 17-H, 22-H, 23-H_b, 28-H, 29-H_{eq}, 30-H), 2.16 (dtd, $^2J_{15b,15a}$ = 14.4, $^3J_{15b,14}$ = 8.1, $^3J_{15b,16}$ = 3.3 Hz, 1 H, 15-H_b), 2.27 (dt, $^2J_{2a,2b}$ = 15.6, $^3J_{2a,3}$ =

7.4 Hz, 1 H, 2-H_a), 2.37 (dt, $^2J_{2b,2a}$ = 15.6, $^3J_{2b,3}$ = 7.4 Hz, 1 H, 2-H_b), 2.51 (s, 3 H, 32-H), 2.68 (dd, $^2J_{31ax,31eq} \approx {}^3J_{31ax,30ax}$ = 12.3 Hz, 1 H, 31-H_{ax}), 2.80–3.01 (m, 4 H, 5-H, 14-H), 3.09 (s, 3 H, 19-H), 3.26 (d, $^2J_{31eq,31ax}$ = 12.3 Hz, 1 H, 31-H_{eq}), 3.32 [m, 1 H, 27-H (under MeOD)], 4.26–4.39 (m, 2 H, 4-H, 16-H), 4.72 (d, $^3J_{21,22}$ = 8.5 Hz, 1 H, 21-H), 7.15 (m, 1 H, 9-H), 7.18–7.27 (m, 4 H, 7-H, 8-H), 7.99 (s, 1 H, 12-H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 11.2, 16.1, 20.3, 20.5, 23.1, 24.9, 25.6, 30.3, 29.8*, 30.7, 31.0, 31.3, 31.8, 34.4, 37.7, 42.0, 43.6, 52.0, 55.6, 56.3, 60.3*, 69.1, 118.2 (q, $^1J_{C,F}$ = 290.9 Hz), 124.4, 127.4, 129.3, 130.4, 139.7, 150.4, 163.1 (q, $^2J_{C,F}$ = 34.3 Hz), 163.2, 171.8, 171.9, 174.7, 181.8 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, MeOD): δ = 1.11 (d, $^3J_{18',17}$ = 6.5 Hz, 3 H, 18′-H), 2.48 (s, 3 H, 32-H), 2.78 (s, 1 H, 19-H), 4.67 (dd, $^3J_{21,22}$ = 8.3 Hz, 1 H, 21-H), 7.95 (s, 1 H, 12-H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 11.7, 16.5, 21.0, 22.9, 38.6, 41.8, 43.8, 55.3, 68.9, 173.9, 179.8 ppm. HRMS (CI): *m*/*z* calcd. for $C_{35}H_{52}N_5O_4S$ [M – OH]$^+$ 638.3740; found 638.3708.

Methyl (2*S*,4*R*)-4-[((*R*)-2-{3-[(*N*-Benzyloxycarbonyl-(*R*)-pipecolyl-(*S*)-isoleucyl)methylamino]-4-methylpentyl}thiazole-4-carbonyl)-amino]-2-methyl-5-phenylpentanoate (24): The free acid **21** (120 mg, 200 μmol) and the hydrochloride **10** (57 mg, 221 μmol) were coupled by the General Procedure for mixed anhydride peptide coupling with the aid of isobutyl chloroformate (28 μL, 216 μmol) and *N*-methylmorpholine (2 × 24 μL, 436 μmol) to give the tetrapeptide **24** (129 mg, 160 μmol, 80%) as a colorless resin after column chromatography (hexane/EtOAc, 1:1, 3:7). R_f = 0.19 (Hex/EtOAc, 1:1). $[\alpha]_D^{20}$ = +19.3 (*c* = 1.0, $CHCl_3$). Major rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.76 (d, $^3J_{19,18}$ = 6.5 Hz, 3 H, 19-H), 0.82 (m, 3 H, 25-H), 0.92 (d, $^3J_{26,23}$ = 6.0 Hz, 3 H, 26-H), 0.95 (d, $^3J_{19',18}$ = 6.5 Hz, 3 H, 19′-H), 1.03 (m, 1 H, 24-H_a), 1.12 (d, $^3J_{10,2}$ = 7.0 Hz, 3 H, 10-H), 1.21–1.65 (m, 7 H, 3-H_a, 24-H_b, 29-H_{ax}, 30-H, 31-H), 1.66–1.91 (m, 3 H, 16-H_a, 18-H, 23-H), 1.99 (ddd, $^2J_{3b,3a}$ = 13.5, $^3J_{3b,2/4}$ = 9.4, $^3J_{3b,2/4}$ = 4.0 Hz, 1 H, 3-H_b), 2.11 (m, 1 H, 16-H_b), 2.29 (d, $^2J_{29eq,29ax}$ = 12.8 Hz, 1 H, 29-H_{eq}), 2.58 (m, 1 H, 2-H), 2.69–3.02 (m, 5 H, 5-H, 15-H, 32-H_{ax}), 2.98 (s, 3 H, 20-H), 3.59 (s, 3 H, 39-H), 4.08 (br. s, 1 H, 32-H_{eq}), 4.29–4.44 (m, 2 H, 4-H, 17-H), 4.80 (dd, $^3J_{22,NH} \approx {}^3J_{22,23}$ = 8.0 Hz, 1 H, 22-H), 4.89 (br. s, 1 H, 28-H), 5.11 (d, $^2J_{34a,34b}$ = 12.0 Hz, 1 H, 34-H_a), 5.16 (d, $^2J_{34b,34a}$ = 12.0 Hz, 1 H, 34-H_b), 6.56 (br. s, 1 H, NH_{Ile}), 7.10–7.38 (m, 11 H, arom. H, NH_{Tup}), 7.88 (s, 1 H, 13-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.0, 15.8, 17.7, 19.6, 20.0, 20.4, 24.1, 24.8*, 25.5*, 29.3, 29.3*, 30.1, 30.2, 36.4, 37.2, 37.8, 41.2, 42.2*, 48.4, 51.6, 53.6, 54.9*, 58.8*, 67.6, 122.3, 126.4, 127.8, 128.1, 128.3, 128.5, 129.4, 136.3, 137.6, 149.8, 156.2*, 160.6, 169.6, 170.4, 173.2, 176.5 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.05 (d, $^3J_{19',18}$ = 6.5 Hz, 3 H, 19′-H), 2.77 (s, 3 H, 20-H), 4.18 (br. s, 1 H, 32-H_{eq}), 4.97 (dd, $^3J_{22,NH}$ = 9.0, $^3J_{22,23}$ = 7.0 Hz, 1 H, 22-H), 5.06 (d, $^2J_{34a,34b}$ = 12.6 Hz, 1 H, 34-H_a), 7.86 (s, 1 H, 13-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2, 16.2, 20.3, 20.5, 23.5, 27.3, 62.7, 122.6 ppm. HRMS (CI): *m*/*z* calcd. for $C_{44}H_{62}N_5O_7S$ [M + H]$^+$ 804.4370; found 804.4331.

(2*S*,4*R*)-4-[((*R*)-2-{3-[Methyl-(*N*-methyl-(*R*)-pipecolyl-(*S*)-isoleucyl)-amino]-4-methylpentyl}thiazole-4-carbonyl)amino]-2-methyl-5-phenylpentanoic Acid, Trifluoroacetic Acid Salt (2): The tetrapeptide **24** (114 mg, 0.142 mmol) was *N*-deprotected with HBr in acetic acid (33 wt.-%, 240 μL, 1.39 mmol) at 0 °C and the resulting hydrobromide was deprotonated with saturated $NaHCO_3$ to afford the free amine. This *N*-deprotected peptide was dissolved in MeOH (1.4 mL), and paraformaldehyde (12.8 mg, 0.142 mmol) was added. After the system had been stirred for 3 h at room temperature, sodium cyanoborohydride (9.4 mg, 0.150 mmol) was added. When the reaction was finished (TLC monitoring), methanol was removed in vacuo and the residue was dissolved in dichloromethane and washed with saturated $NaHCO_3$. The aqueous layer was extracted with dichloromethane and the combined organic layers were dried with Na_2SO_4. The *N*-methylated tetrapeptide (64 mg, 93.6 μmol, 66%) was obtained after flash chromatography (CH_2Cl_2/MeOH, 99:1, 98:2, 95:5, 9:1) as a yellow oil. A mixture of the *N*-methylated tetrapeptide (39 mg, 57.0 μmol) and NaOH (1 M, 172 μL, 172 μmol) in dioxane (0.57 mL) was heated to 80 °C until complete saponification. The solvent was evaporated in vacuo, and the residue was dissolved in water, acidified to pH 2 with trifluoroacetic acid, and extracted twice with EtOAc. The combined organic layers were dried with Na_2SO_4 and the solvent was evaporated in vacuo. Purification by flash chromatography (CH_2Cl_2/MeOH, 95:5, 9:1) gave **2** (44 mg, 56.1 μmol) as white solid in quantitative yield. R_f = 0.11 (CH_2Cl_2/MeOH, 9:1); m.p. 60–62 °C. $[\alpha]_D^{20}$ = –17.1 (*c* = 1.0, MeOH). Major rotamer: ^{1}H NMR (400 MHz, MeOD): δ = 0.81 (d, $^3J_{19,18}$ = 6.3 Hz, 3 H, 19-H), 0.93 (t, $^3J_{25,24}$ = 7.1 Hz, 3 H, 25-H), 0.99 (d, $^3J_{19',18}$ = 6.3 Hz, 3 H, 19′-H), 1.02 (d, $^3J_{26,23}$ = 6.5 Hz, 3 H, 26-H), 1.23 (m, 1 H, 24-H_a), 1.53–1.86 (m, 6 H, 3-H_a, 18-H, 24-H_b, 29-H_{ax}, 30-H_{ax}, 31-H_{ax}), 1.86–2.08 (m, 5 H, 3-H_b, 16-H_a, 23-H, 30-H_{eq}, 31-H_{eq}), 2.11–2.27 (m, 2 H, 16-H_b, 29-H_{eq}), 2.56 (m, 1 H, 2-H), 2.74 (s, 3 H, 33-H), 2.81–3.02 (m, 4 H, 5-H, 15-H), 3.09 (dd, $^2J_{32ax,32eq} \approx {}^3J_{32ax,31ax}$ = 12.3 Hz, 1 H, 32-H_{ax}), 3.11 (s, 3 H, 20-H), 3.49 (d, $^2J_{32eq,32ax}$ = 11.5 Hz, 1 H, 32-H_{eq}), 3.76 (d, $^3J_{28,29ax}$ = 10.8 Hz, 1 H, 28-H), 4.24–4.47 (m, 2 H, 4-H, 17-H), 4.70 (d, $^3J_{22,23}$ = 7.8 Hz, 1 H, 22-H), 7.16 (m, 1 H, 9-H), 7.19–7.29 (m, 4 H, 7-H, 8-H), 7.95 (s, 1 H, 12-H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 11.3, 16.0, 18.5, 20.3, 20.5, 22.3, 24.0, 25.5, 30.2, 30.3*, 30.9, 31.4, 37.5, 37.9, 39.2, 42.4, 42.9, 50.7, 56.0, 56.2, 60.6*, 68.1, 118.2 (q, $^1J_{C,F}$ = 290.6 Hz), 124.0, 127.4, 129.3, 130.4, 139.5, 150.5, 163.1 (q, $^2J_{C,F}$ = 32.8 Hz), 163.1, 169.2, 171.9, 174.6, 180.0 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, MeOD): δ = 2.78 (s, 1 H, 20-H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 11.8, 16.4, 18.4, 31.2, 42.1, 43.1, 178.5 ppm. HRMS (CI): *m*/*z* calcd. for $C_{36}H_{56}N_5O_5S$ [M + H]$^+$ 670.4002; found 670.3984.

((*R*)-2-{3-[Methyl-(*N*-methyl-(*R*)-pipecolyl-(*S*)-isoleucyl)amino]-4-methylpentyl}thiazole-4-carbonyl)(*S*)-phenylalanine, Trifluoroacetic Acid Salt (25): The free acid **21** (120 mg, 200 μmol) and L-phenylalanine methyl ester hydrochloride (47 mg, 218 μmol) were coupled by the General Procedure for mixed anhydride peptide coupling with the aid of isobutyl chloroformate (28 μL, 216 μmol) and *N*-methylmorpholine (2 × 24 μL, 436 μmol) to give the protected tetrapeptide (136 mg, 178 μmol, 89%) as a white foam after column chromatography (hexane/EtOAc, 1. 1:1, 2. 4:6, 3. 3:7). This tetrapeptide (99 mg, 0.130 mmol) was *N*-deprotected with HBr in acetic acid (33 wt.-%, 180 μL, 1.04 mmol) at 0 °C and the resulting hydrobromide was deprotonated with saturated $NaHCO_3$ to afford the free amine. The *N*-deprotected peptide was dissolved in MeOH (1.3 mL) and paraformaldehyde (11.8 mg, 0.131 mmol) was added. After the system had been stirred for 3 h at room temperature, sodium cyanoborohydride (9.0 mg, 0.143 mmol) was added. When the reaction was finished (TLC monitoring), methanol was removed in vacuo and the residue was dissolved in dichloromethane and washed with saturated $NaHCO_3$. The aqueous layer was extracted with dichloromethane and the combined organic layers were dried with Na_2SO_4. The *N*-methylated tetrapeptide (40 mg, 62.3 μmol, 48%) was obtained after flash chromatography (CH_2Cl_2/MeOH, 1. 99:1, 2. 98:2, 3. 9:1) as a yellow oil. A mixture of the *N*-methylated tetrapeptide (40 mg, 62.3 μmol) and NaOH (1 M, 100 μL, 100 μmol) in dioxane (0.62 mL) was stirred at 0 °C until complete saponification. The solvent was evaporated in vacuo, and the residue was dissolved in water, acidified to pH 2 with trifluoroacetic acid, and extracted twice with EtOAc. The combined

organic layers were dried with Na_2SO_4 and the solvent was evaporated in vacuo. Purification by flash chromatography (CH_2Cl_2/MeOH, 9:1) gave **25** as a white solid in quantitative yield. R_f = 0.10 (CH_2Cl_2/MeOH, 9:1); m.p. 88 °C. $[\alpha]_D^{20}$ = –1.0 (*c* = 1.0, MeOH). Major rotamer: ^{1}H NMR (400 MHz, MeOD): δ = 0.79 (d, $^3J_{16,15}$ = 6.5 Hz, 3 H, 16-H), 0.91 (t, $^3J_{22,21}$ = 7.4 Hz, 3 H, 22-H), 0.97 (d, $^3J_{16',15}$ = 6.5 Hz, 3 H, 16′-H), 1.01 (d, $^3J_{23,20}$ = 6.8 Hz, 3 H, 23-H), 1.21 (ddq, $^2J_{21a,21b}$ = 13.6, $^3J_{21a,20}$ = 9.4, $^3J_{21a,22}$ = 7.3 Hz, 1 H, 21-H_a), 1.52–1.69 (m, 2 H, 21-H_b, 27-H_{ax}), 1.71–2.05 (m, 7 H, 13-H_a, 15-H, 20-H, 26-H_{ax}, 27-H_{eq}, 28-H), 2.10–2.27 (m, 2 H, 26-H_{eq}, 13-H_b), 2.73 (s, 3 H, 30-H), 2.80–3.00 (m, 2 H, 12-H_a, 12-H_b), 3.06 (m, 1 H, 29-H_{ax}), 3.07 (s, 3 H, 17-H), 3.20 (dd, $^2J_{3a,3b}$ = 13.8, $^3J_{3a,2}$ = 6.8 Hz, 1 H, 3-H_a), 3.33 [m, 1 H, 3-H_b (under MeOD)], 3.48 (d, $^2J_{29eq,29ax}$ = 12.3 Hz, 1 H, 29-H_{eq}), 3.78 (d, $^3J_{25,26ax}$ = 11.6 Hz, 1 H, 25-H), 4.21 (br. s, 1 H, 14-H), 4.69 (d, $^3J_{19,20}$ = 7.5 Hz, 1 H, 19-H), 4.85 [m, 1 H, 2-H (under H_2O)], 7.15–7.30 (m, 5 H, arom. H), 8.04 (s, 1 H, 10-H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 11.4, 16.1, 20.3, 20.5, 22.3, 24.0, 25.4, 30.0, 30.2, 30.4*, 31.0, 31.2, 37.5, 38.5, 42.8, 55.2, 56.1, 60.9*, 68.1, 118.2 (q, $^1J_{C,F}$ = 289.5 Hz), 124.6, 127.9, 129.4, 130.5, 138.2, 150.0, 162.7, 163.0 (q, $^2J_{C,F}$ = 32.8 Hz), 169.2, 172.2, 174.4, 174.7 ppm. Selected signals of the minor rotamer: ^{1}H NMR (400 MHz, MeOD): δ = 1.09 (d, $^3J_{16',15}$ = 6.3 Hz, 3 H, 16′-H), 2.34 (m, 1 H, 13-H_b), 2.55 (s, 3 H, 30-H), 2.65 (s, 3 H, 17-H), 3.70 (dd, $^3J_{14,13a/b} \approx {}^3J_{14,15}$ = 9.0 Hz, 1 H, 14-H), 3.84 (d, $^3J_{25,26ax}$ = 12.0 Hz, 1 H, 25-H), 7.97 (s, 1 H, 10-H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 11.7, 16.5, 20.6, 20.9, 24.5, 28.3, 38.7, 43.0, 55.3, 64.8, 124.8, 127.8, 129.3, 130.6, 138.5, 150.2, 169.4, 172.6, 173.7 ppm. HRMS (CI): *m*/*z* calcd. for $C_{24}H_{42}N_5O_3S$ [M – C_9H_7O]$^+$ 480.3008; found 480.2983.

MTT Assay: Cells were seeded at 6×10^3 cells per well on 96-well plates (Corning CellBind®) in complete medium (180 μL) and directly treated with tubulysins and pretubulysins dissolved in methanol in serial dilution. Each compound, as well as the internal methanol control, was tested in duplicate. After 5 d incubation, MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide, 5 mg mL^{-1}, 20 μL] in PBS (phosphate-buffered saline, pH 7.4) was added to each well and incubation was continued for a further 2 h at 37 °C. The medium was then discarded and cells were washed with PBS (100 μL) before addition of propan-2-ol/10 N HCl (250:1, 100 μL) in order to dissolve formazan granules. The absorbance at 570 nm was measured with a microplate reader (EL808, Bio-Tek Instruments Inc.), and cell viability was expressed as a percentage relative to the corresponding methanol control. IC_{50} values were obtained by sigmoidal curve fitting.

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