DOI: 10.1002/ejoc.201100155

Syntheses and Evaluation of Simplified Pretubulysin Analogues

Jens L. Burkhart,[a] Rolf Müller,[b] and Uli Kazmaier*[a]

Keywords: Natural products / Antitumor agents / Synthesis design / Myxobacteria / Tubulysins

The syntheses of new tubulysin analogues are described, in which the central amino acid, tubuvaline, is replaced by the rather simple building blocks phenyltubuvaline and phenoxytubuvaline. These analogues can be obtained in only two to three steps from easily accessible starting materials. Although the new derivatives are less active than the tubulysins, their activities towards U-2 OS tumor cells are still in the nanomolar range.

Introduction

The development of selective anticancer drugs, able to discriminate between normal and tumor cells, is an important goal in modern cancer therapy. The highly promising approach to target tumor cells with monoclonal antibodies, loaded with a suitable antitumor drug, requires highly potent drugs.[1] Aside from the maytansanoids[2] and epothilones,[3] the peptides auristatine[4] and dolastatine 10[5] and the family of the tubulysins[3,6] (Figure 1) are excellent candidates for this approach, based on their high biological activity. The tubulysins, isolated by Höfle and co-workers from myxobacteria,[7] especially *Angiococcus disciformis* An d48 and *Archangium gephyra* Ar 315, belong to a nine-membered group of linear tetrapeptides (tubulysins A–I).[7] Very recently, further derivatives were discovered in *A. disciformis* An d48 and *Cystobacter* SBCb004 by the Müller group.[8] Common to all members of the tubulysin family is their linear structure, beginning with an *N*-terminal *N*-methylated D-pipecolinic acid (D-Mep), followed by the only proteinogenic amino acid L-Ile. The next building block is called tubuvaline (Tuv), an unusual thiazole amino

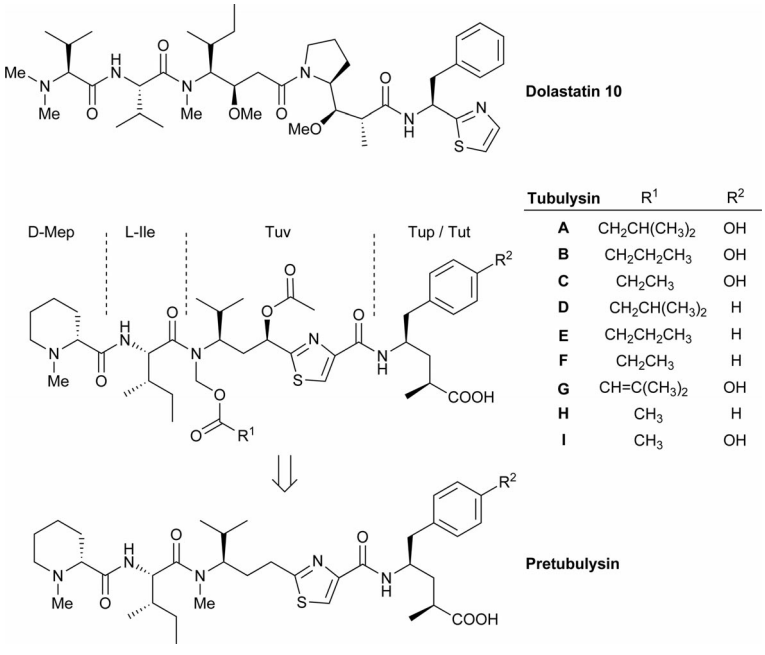


Figure 1. Peptidic tubulin polymerization inhibitors.

[a] Institute for Organic Chemistry, Saarland University,
P.O. Box 151150, 66041 Saarbrücken, Germany
Fax: +49-681-302-2409
E-mail: u.kazmaier@mx.uni-saarland.de
[b] Department of Pharmaceutical Biotechnology,
Saarland University und Helmholtz-Institut für Pharmazeutische Forschung Saarland,
P.O. Box 151150, 66041 Saarbrücken, Germany
Fax: +49-681-302-70202
E-mail: rom@mx.uni-saarland.de

acid, bound through an exotic acylated N,O-acetal structure. The natural tubulysins differ mainly in the side chain of the N,O-acetal group. An α-methylated aromatic γ-amino acid is the *C*-terminal amino acid. In some tubulysins, this amino acid is derived from phenylalanine (tubulysins D–F, and H), in others from tyrosin (tubulysins A–C, and G). The newly explored derivatives mainly differ in the oxidation and acylation state of the Tuv fragment and are probably biosynthetic intermediates.[9] Pretubulysin was found to be a central intermediate of the tubuphenlyalanine tubulysins D–F and H.[10]

The tubulysins belong to a group of natural products that interact with the eucaryotic cytoskeleton by binding to microtubuli. Inhibition of tubulin polymerization induces apoptosis.[6,11] This mode of action differs from that of paclitaxel and the epothilones. The tubulysins also show a higher cytotoxicity towards a wide range of tumor cells in the low nano- or even picomolar range.[7a] From a structural point of view, the tubulysins are in part very similar to dolastatine 10, in particular, the left-hand side of the molecule is conserved. A tertiary amine is found at the N terminus of both types of peptides, followed by an apolar amino acid (Val or Ile), and an *N*-alkylated apolar unusual amino acid building block. On the C terminus both natural products contain phenylalanine-derived unusual amino acids. The major difference is found in the area between the sterically demanding side chain of the *N*-alkylated amino acid and the *C*-terminal building block. Both natural products probably interact with the same binding side at the vinca domain of the tubulin. Recent NMR spectroscopic studies by the Carlomagno group indicated that the conformations of these tubulin-bound peptides were very similar and that the variable part in between the apolar left-hand side and the aromatic substituent probably acted as a spacer to bring the interacting parts of the natural products into the correct orientation for binding.[12]

At first, the tubulysins seem to be relatively simple structures, especially with respect to other antitumor drugs, such as paclitaxel. Nevertheless, to date, only two total syntheses have been described in the literature. In 2006, the Ellman group reported the first synthesis of tubulysin D,[13] and recently Wessjohann et al. described the first synthesis of tubulysin B.[14] In addition, the groups of Höfle[15] and Dömling[16] patented syntheses of tubulysins. Very recently, Tamura et al. reported the conversion of tubulysin D into a cyclic derivative, bridging the N terminus of Tuv with an OH group α to the thiazole ring.[17] Probably the major problem during the total synthesis is the introduction of the N,O-acetal side chain found on the N terminus of the Tuv, which is found in all tubulysins. With respect to an application as an anticancer drug, this rather labile structure should also be a critical candidate for enzymatic cleavage.

Therefore, besides the total syntheses of the natural products, a range of syntheses towards tubulysin analogues[18] and building blocks[19] have also been reported, as well as detailed structure–activity (SAR) studies. These studies clearly indicate that the N,O-acetal is not necessary for high biological activity.[13,20] Compounds incorporating a simple *N*-methylamide at the same position can be equally potent,[21] a significant drop in activity is only observed when the N substituent is removed completely .[22] SAR studies have also focused on the *N*-terminal amino acid, *N*-methyl pipecolinic acid (Mep), which can probably be replaced by *N*-methylsarcosine, and the *C*-terminal tubuphenylalanine (Tup), which can be substituted with a wide range of alternative functionalities, with retention of biological activity.[21a] These studies are consistent with the binding studies reported by Carlomagno et al.[12]

Results and Discussion

As a result of biosynthetic studies, we postulated that pretubulysin (Figure 1) was a common biosynthetic intermediate.[10] When considering that the rather labile N,O-acetal side chains in the Tuv fragment are missing, this compound should be an ideal candidate to develop tubulysin drugs. Especially because the biological activity of pretubulysin is only slightly lower than tubulysin itself and still in the low nanomolar range.[23] To simplify the structure even more, we decided to replace the thiazole moiety with a simple phenyl ring. In principle, this should allow the introduction of a wide range of functionalities in this area to modulate, for example, the pharmacokinetic properties of this class of compound. With respect to the lack of a Tuv acetoxy group in pretubulysin, the removal of the stereogenic center α to the heterocycle should also allow us to replace the remaining CH_2 bond with other functionalities, such as an ether bridge. Herein, we describe the synthesis and biological activity of two new derivatives of pretubulysin, which we call phenylpretubulysin (**1**) and phenoxypretubulysin (**2**) (Figure 2).

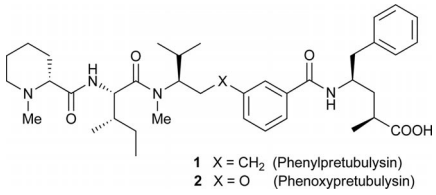
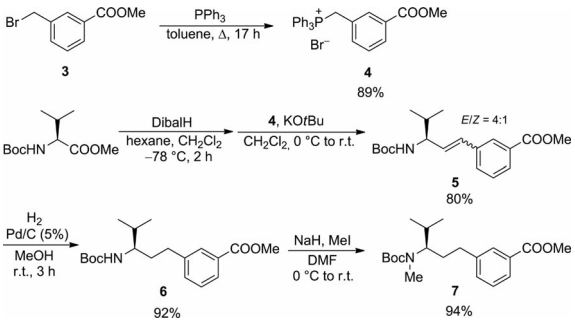


Figure 2. Pretubulysin derivatives **1** and **2**.

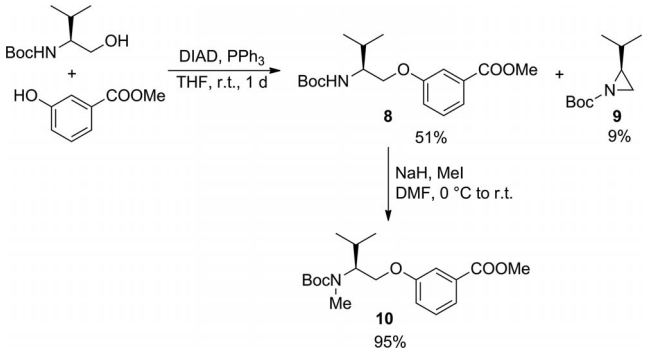
The synthesis of the phenylpretubulysin intermediate **7** (phenylpretubuvaline) is shown in Scheme 1. The starting point was bromide **3**, which was obtained by radical bromination of methyl *m*-toluate.[24] Reaction with PPh_3 under standard conditions provided phosphonium salt **4**,[25] which was subjected to a Wittig reaction with *tert*-butyloxycarbonyl (Boc-)protected valinal. The Wittig reaction was carried out as a one-pot protocol by adding the Wittig salt to a freshly prepared solution of the aldehyde in CH_2Cl_2. The required unsaturated derivative **5** was obtained in good yield as a 4:1 mixture of *E*/*Z* isomers. In our case, the olefin geometry did not play any role because the double bond was removed in the next step by catalytic hydrogenation to form **6**. Subsequent *N*-methylation provided the protected phenylpretubuvaline **7**. Attempts to introduce the *N*-methyl

group at an earlier stage were less successful. If the one-pot sequence of diisobutylaluminum hydride (DIBALH) reduction/Wittig olefination was carried out with the *N*-methylvaline derivative, the yield dropped to 44% and gave rise to the unsaturated phenylpretubuvaline as a 1:1 isomeric mixture (*E*/*Z*).

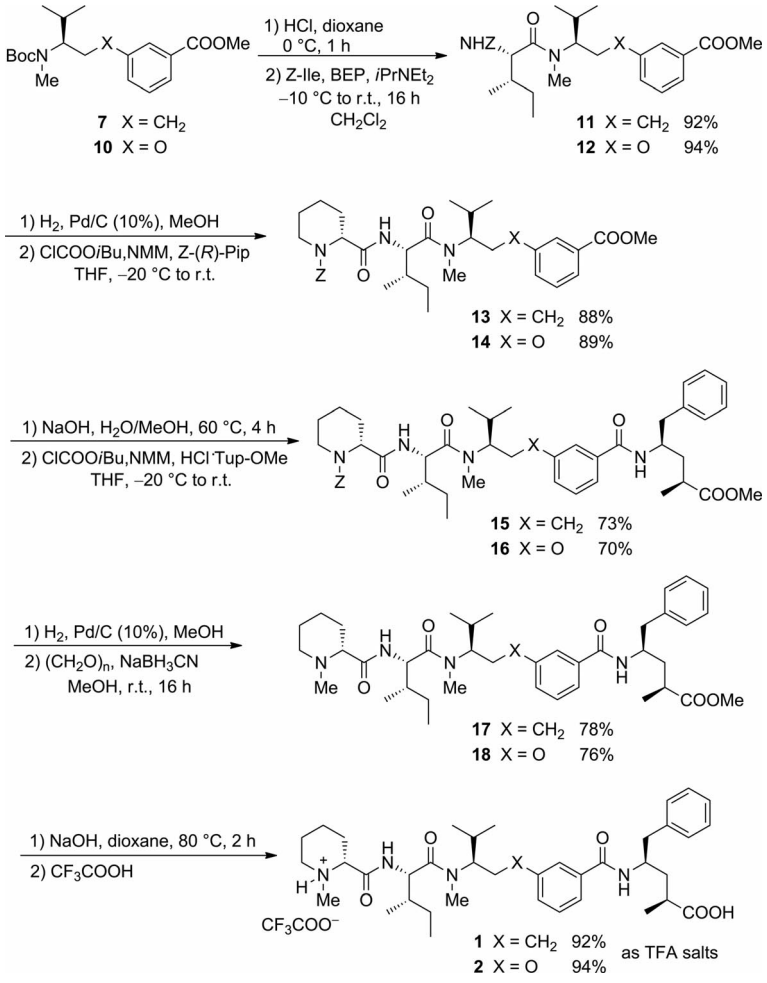


Scheme 1. Synthesis of phenylpretubuvaline **7**.

The synthesis of the corresponding phenoxypretubuvaline derivative **10** (Scheme 2) started with enantiomerically pure Boc-valinol,[26] which was treated with methyl *m*-hydroxybenzoate under Mitsunobu conditions.[27] The coupling product **8** was obtained in moderate yield, but excellent optical purity (99% *ee*), accompanied by a small amount of aziridine **9**.[28] Even increasing the reaction time to three days did not improve the yield. Subsequent *N*-methylation occurred in nearly quantitative yield. Unsurprisingly, the attempt to obtain **10** directly by the Mitsunobu reaction using protected *N*-methylvalinol failed completely. It is likely that nucleophilic attack of the phenolate on the activated valinol derivative is also the critical step in the former reaction.



Scheme 2. Synthesis of phenoxypretubuvaline **10**.



Scheme 3. Synthesis of phenylpretubulysin (**1**) and phenoxypretubulysin (**2**). TFA = trifluoroacetate.

With these building blocks in hand, the synthesis of the pretubulysin derivatives **1** and **2** was accomplished according to Scheme 3. Cleavage of the Boc-protecting group and coupling with Z-Ile gave rise to the required dipeptides **11** and **12**. 2-Bromo-1-ethylpyridinium tetrafluoroborate (BEP) was used as a coupling reagent to avoid racemization, which has frequently been observed in couplings of *N*-methyl amino acids, and to achieve high yields.[29] Subsequent removal of the protecting groups and coupling with the *N*-terminal (*R*)-pipecolinic acid (Pip) and the *C*-terminal tubuphenylalanine (Tup) derivative gave rise to tetrapeptides **15** and **16**. Removal of the benzyloxycarbonyl (Z)-protecting group and reductive methylation followed by saponification of the ester moiety provided the required pretubulysin derivatives **1** and **2** in a straightforward manner.

The biological activity of our new pretubulysin derivatives **1** and **2**, as well as methyl ester **17**, were tested, and compared with tubulysins A and D as well as pretubulysin, towards human bone osteosarcoma (U-2 OS) cells (Table 1).[23] Whereas the tubulysins and pretubulysins showed IC_{50} values in the sub-nanomolar range, phenylanalogue **1** was two magnitudes of order less potent (ca. 100 nM). Methyl ester **17**, which was probably a prodrug, and the phenoxy derivative **2** showed a fourfold lower activity (Table 1).

Table 1. Cytotoxicity of tubulysins and analogues towards U-2 OS cells.

	IC_{50} [ng mL^{-1}]
Tubulysin A	0.19
Tubulysin D	0.034
Pretubulysin D	0.56
Phenylpretubulysin (**1**)	78.6
Phenoxypretubulysin (**2**)	318.2
Methyl ester **17**	284.6

Conclusions

We have shown that the complex structure of tubulysins can be significantly reduced by replacing the Tuv subunit with a relatively simple spacer unit. Although the activity is significantly lower than that of the natural products and pretubulysin, the activity is still in the nanomolar range. Therefore, these compounds might be interesting for further developments, especially based on their easy synthetic availability.

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 EtOAc and hexanes were generally used as eluents. Analysis by TLC was carried out on commercially precoated Polygram SIL-G/UV 254 plates (Machery–Nagel, Düren). Visualization was accomplished with UV light, $KMnO_4$ solution, or ninhydrin. ^{1}H and ^{13}C NMR spectra were obtained at room temperature with a Bruker AV 400 spectrometer. Chemical shifts are expressed in ppm relative to internal solvent. Selected signals of minor isomers were extracted from the NMR spectra of the isomeric mixtures. The enantio- and diastereomeric ratios were determined by HPLC on a Shimadzu 10A VP using a chiral column (Chiralcel OD-H) and an achiral silica gel column (LiChrosorb 5 μ Si-60 A). Optical rotation measurements were performed on 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 on a Finnigan MAT 95Q instrument by using the CI technique. Elemental analyses were performed on a Leco CHN900 instrument.

Methyl (*S*)-3-[3-(*tert*-Butoxycarbonylamino)-4-methylpent-1-enyl]-benzoate (5): A solution of DIBALH (1 M) in hexane (9.00 mL, 9.00 mmol) was added dropwise at –78 °C to a solution of Boc-protected methyl valinate (1.02 g, 4.41 mmol) in dry dichloromethane (25 mL). After stirring the reaction mixture for 2 h at –78 °C, phosphonium salt **4**[25] (2.38 g, 4.84 mmol) and KO*t*Bu (544 mg, 4.85 mmol) were added and the reaction was warmed to room temperature overnight. The reaction mixture was poured into 10% aqueous tartaric acid (100 mL) and vigorously stirred for 30 min. After separation of the layers, the aqueous layer was extracted thrice with EtOAc. The combined organic layers were washed with water and brine, dried with Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (hexane/EtOAc, 9:1) to yield **5** (1.18 g, 3.54 mmol, 80%) as a colorless oil and a mixture of *E* and *Z* isomers (*E*/*Z* = 4:1). R_f = 0.29 (hexane/EtOAc, 8:2). **(*E*)-5:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.96 (d, $^3J_{13,12}$ = 6.8 Hz, 3 H, 13-H), 0.97 (d, $^3J_{13',12}$ = 6.8 Hz, 3 H, 13′-H), 1.47 (s, 9 H, 16-H), 1.87 (m, 1 H, 12-H), 3.93 (s, 3 H, 1-H), 4.15 (br. s, 1 H, 4-H), 4.63 (br. s, 1 H, NH), 6.17 (dd, $^3J_{10,9}$ = 15.9, $^3J_{10,11}$ = 6.5 Hz, 1 H, 10-H), 6.53 (d, $^3J_{9,10}$ = 15.9 Hz, 1 H, 9-H), 7.38 (dd, $^3J_{5,6}$ = $^3J_{5,4}$ = 7.7 Hz, 1 H, 5-H), 7.53 (d, $^3J_{4,5}$ = 7.8 Hz, 1 H, 4-H), 7.89 (d, $^3J_{6,5}$ = 7.8 Hz, 1 H, 6-H), 8.05 (s, 1 H, 8-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.3 (q, C-13), 18.8 (q, C-13′), 28.4 (q, C-16), 32.7 (d, C-12), 52.1 (q, C-1), 57.7, (d, C-11), 79.4 (s, C-15), 127.2 (d, C-8), 128.4 (d, C-6), 128.6 (d, C-5), 129.7 (d, C-9), 130.4 (s, C-3), 130.6 (d, C-10), 130.8 (s, C-4), 137.3 (s, C-7), 155.5 (s, C-14), 167.0 (s, C-2) ppm. **(*Z*)-5:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (d, $^3J_{13,12}$ = 6.6 Hz, 6 H, 13-H, 13′-H), 1.42 (s, 9 H, 16-H), 1.78 (m, 1 H, 12-H), 3.91 (s, 3 H, 1-H), 4.40 (br. s, 1 H, 4-H), 4.53 (br. s, 1 H, NH), 5.55 (dd, $^3J_{10,9}$ = 11.8, $^3J_{10,11}$ = 6.5 Hz, 1 H, 10-H), 6.54 (d, $^3J_{9,10}$ = 9.6 Hz, 1 H, 9-H), 7.41 (dd, $^3J_{5,6}$ = $^3J_{5,4}$ = 7.7 Hz, 1 H, 5-H), 7.64 (br. s, 1 H, 4-H), 7.91 (d, $^3J_{6,5}$ = 7.8 Hz, 1 H, 6-H), 7.94 (s, 1 H, 8-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.2 (q, C-13), 18.4 (q, C-13′), 28.3 (q, C-16), 33.4 (d, C-12), 52.1 (q, C-1), 53.4, (d, C-11), 79.1 (s, C-15), 128.1 (d, C-6), 128.5 (d, C-5), 129.9 (d, C-4), 130.1 (d, C-9), 130.2 (s, C-3), 132.1 (d, C-10), 133.0 (s, C-8), 137.0 (s, C-7), 155.2 (s, C-14), 167.0 (s, C-2) ppm. $C_{19}H_{27}NO_4$ (333.42): calcd. C 68.44, H 8.16, N 4.20; found C 68.43, H 8.54, N 4.20. HRMS (CI): calcd. for $C_{15}H_{20}NO_4$ [M – C_4H_7]$^+$ 278.1392; found 278.1380.

Methyl (*R*)-3-[3-(*tert*-Butoxycarbonylamino)-4-methylpentyl]benzoate (6): A solution of **5** (513 mg, 1.53 mmol, *E*/*Z* mixture) in MeOH (7 mL) was stirred under hydrogen in the presence of 5% Pd/C (50 mg) until the reduction was complete (3 h). After filtration through Celite, the solvent was removed in vacuo and the crude product was purified by flash chromatography (hexane/EtOAc, 8:2) to yield **6** (470 mg, 1.40 mmol, 92%) as a white solid; m.p. 72–73 °C. R_f = 0.31 (hexane/EtOAc, 8:2). HPLC: Chiracel OD-H (250 × 4.6), hexane/2-propanol = 95:5, 1 mL min^{-1}, t_R[(*R*)-**6**] = 6.61 min, t_R[(*S*)-**6**] = 8.19 min. $[\alpha]_D^{21}$ = –7.6 (*c* = 1.0, *ee* = 99%,

$CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 0.87 (d, $^3J_{13,12}$ = 6.8 Hz, 3 H, 13-H), 0.90 (d, $^3J_{13',12}$ = 6.8 Hz, 3 H, 13′-H), 1.45 (s, 9 H, 16-H), 1.56 (m, 1 H, 10-H_a), 1.69–1.83 (m, 2 H, 10-H_b, 12-H), 2.67 (m, 1 H, 9-H_a), 2.75 (m, 1 H, 9-H_b), 3.50 (m, 1 H, 11-H), 3.90 (s, 3 H, 1-H), 4.35 (d, $^3J_{NH,11}$ = 9.4 Hz, 1 H, NH), 7.32–7.39 (m, 2 H, 5-H, 6-H), 7.84–7.86 (m, 2 H, 4-H, 8-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.6 (q, C-13), 19.1 (q, C-13′), 28.4 (q, C-16), 32.4 (d, C-12), 32.6 (t, C-9), 34.7 (t, C-10), 52.0 (q, C-1), 55.4, (d, C-11), 79.0 (s, C-15), 127.1 (d, C-4), 128.4 (d, C-5), 129.4 (d, C-8), 130.2 (s, C-3), 133.1 (d, C-6), 142.5 (s, C-7), 156.0 (s, C-14), 167.2 (s, C-2) ppm. $C_{19}H_{29}NO_4$ (335.44): calcd. C 68.03, H 8.71, N 4.18; found C 68.02, H 8.63, N 4.20. HRMS (CI): calcd. for $C_{19}H_{30}NO_4$ $[M + H]^+$ 336.2175; found 336.2169.

Methyl (*R*)-3-{3-[(*tert*-Butoxycarbonyl)methylamino]-4-methylpentyl}benzoate (7): Methyl iodide (280 μL, 4.50 mmol) was added to a solution of **6** (373 mg, 1.11 mmol) in anhydrous DMF (8 mL). After cooling to 0 °C, NaH (60% in oil, 102 mg, 2.50 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 20 h before it was quenched with water, acidified to pH 6 with saturated NH_4Cl, and extracted with EtOAc. The combined organic layers were washed twice with water, 5% $Na_2S_2O_3$, water, and brine. The organic phase was dried with Na_2SO_4 and concentrated. The crude material was purified by flash chromatography (hexane/EtOAc, 8:2) to yield **7** (380 mg, 1.09 mmol, 98%) as a colorless oil and a mixture of rotamers. R_f = 0.33 (hexane/EtOAc, 8:2). $[\alpha]_D^{21}$ = –7.5 (*c* = 0.9, $CHCl_3$). **Major rotamer:** ^1H NMR (400 MHz, $CDCl_3$): δ = 0.83 (d, $^3J_{13,12}$ = 6.6 Hz, 3 H, 13-H), 0.90 (d, $^3J_{13',12}$ = 6.6 Hz, 3 H, 13′-H), 1.43 (s, 9 H, 16-H), 1.57–1.67 (m, 2 H, 10-H_a, 12-H), 1.91 (m, 1 H, 10-H_b), 2.55 (m, 2 H, 9-H), 2.65 (s, 3 H, 17-H), 3.82 (m, 1 H, 11-H), 3.90 (s, 3 H, 1-H), 7.31–7.38 (m, 2 H, 5-H, 6-H), 7.83–7.87 (m, 2 H, 4-H, 8-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.1 (q, C-13), 20.3 (q, C-13′), 28.1 (q, C-17), 28.4 (q, C-1), 30.6 (d, C-12), 31.7 (t, C-10), 32.7 (t, C-9), 52.0 (q, C-1), 60.5 (d, C-11), 79.3 (s, C-15), 127.1 (d, C-4), 128.4 (d, C-5), 129.0 (d, C-8), 130.2 (s, C-3), 133.1 (d, C-6), 142.7 (s, C-7), 156.6 (s, C-14), 167.2 (s, C-2) ppm. **Minor rotamer:** ^1H NMR (400 MHz, $CDCl_3$): δ = 0.84 (d, $^3J_{13,12}$ = 6.7 Hz, 3 H, 13-H), 0.92 (d, $^3J_{13',12}$ = 6.6 Hz, 3 H, 13′-H), 1.47 (s, 9 H, 16-H), 2.72 (s, 3 H, 17-H), 3.59 (br. s, 1 H, 11-H), 3.90 (s, 3 H, 1-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.6 (q, C-13), 19.9 (q, C-13′), 28.5 (q, C-1), 30.9 (d, C-12), 31.5 (t, C-10), 32.5 (t, C-9), 52.0 (q, C-1), 79.0 (s, C-15), 127.1 (d, C-4), 128.3 (d, C-5), 130.1 (s, C-3), 133.0 (d, C-6), 142.4 (s, C-7), 167.2 (s, C-2) ppm. $C_{20}H_{31}NO_4$ (349.46): calcd. C 68.74, H 8.94, N 4.01; found C 68.46, H 8.97, N 4.32. HRMS (CI): calcd. for $C_{20}H_{32}NO_4$ $[M + H]^+$ 350.2331; found 350.2378.

Methyl (*S*)-3-[2-(*tert*-Butoxycarbonylamino)-3-methylbutoxy]benzoate (8): Diisopropyl azodicarboxylate (DIAD) (112 μL, 0.53 mmol, 94% purity) was added to a solution of Boc-protected valinol (116 mg, 57 mmol), methyl 3-hydroxybenzoate (81 mg, 0.53 mmol), and triphenylphosphane (140 mg, 0.53 mmol) in dry THF (2 mL) at 0 °C. The reaction mixture was stirred for 24 h and then concentrated in vacuo. The crude product was purified by flash chromatography (hexane/EtOAc, 9:1, 8:2) to yield of **8** (90 mg, 0.27 mmol, 51%) as a colorless oil. R_f = 0.32 (hexane/EtOAc, 8:2). HPLC: Chiracel OD-H (250 × 4.6), hexane/EtOH = 99:1, 1 mL min^{-1}, t_R[(*R*)-**8**] = 11.55 min, t_R[(*S*)-**8**] = 12.64 min. $[\alpha]_D^{20}$ = –60.7 (*c* = 0.7, *ee* = 99%, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 0.98 (d, $^3J_{12,11}$ = 6.8 Hz, 3 H, 12-H), 0.99 (d, $^3J_{12',11}$ = 6.8 Hz, 3 H, 12′-H), 1.45 (s, 9 H, 15-H), 2.02 (m, 1 H, 11-H), 3.74 (br. s, 1 H, 10-H), 3.99 (s, 3 H, 1-H), 4.06 (dd, $^2J_{9a,9b}$ = 9.3, $^3J_{9a,10}$ = 3.2 Hz, 1 H, 9-H_a), 4.10 (dd, $^2J_{9b,9a}$ = 9.3, $^3J_{9b,10}$ = 4.0 Hz, 1 H, 9-H_b), 4.79 (d, $^3J_{NH,10}$ = 9.0 Hz, 1 H, NH), 7.09 (ddd, $^3J_{6,5}$ = 8.2, $^4J_{6,8}$ = 2.6, $^4J_{6,4}$ = 0.8 Hz, 1 H, 6-H), 7.34 (dd, $^3J_{5,6}$ = $^3J_{5,4}$ = 8.0 Hz, 1 H, 5-H), 7.54 (dd, $^4J_{8,6}$ = 2.2, $^4J_{8,4}$ = 1.5 Hz, 1 H, 8-H), 7.63 (ddd, $^3J_{4,5}$ = 7.8, $^4J_{4,6}$ = $^4J_{4,8}$ = 1.1 Hz, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.7 (q, C-12), 19.6 (q, C-12′), 28.4 (q, C-15), 29.5 (d, C-11), 52.2 (q, C-1), 55.2 (d, C-10), 68.4 (t, C-9), 79.3 (s, C-14), 114.8 (d, C-8), 119.7 (d, C-6), 122.2 (d, C-4), 129.4 (d, C-5), 131.5 (s, C-3), 155.7 (s, C-13), 158.7 (s, C-7), 166.9 (s, C-2) ppm. $C_{18}H_{27}NO_5$ (337.41): calcd. C 64.07, H 8.07, N 4.15; found C 64.01, H 7.81, N 3.75. HRMS (CI): calcd. for $C_{18}H_{28}NO_5$ $[M + H]^+$ 338.1967; found 338.1962.

Methyl (*S*)-3-{2-[(*tert*-Butoxycarbonyl)methylamino]-3-methylbutoxy}benzoate (10): To a solution of **8** (551 mg, 1.63 mmol) in anhydrous DMF (10 mL) methyl iodide (410 μL, 6.59 mmol) was added. After cooling to 0 °C NaH (60% in oil) (170 mg, 4.25 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 21 h, before it was quenched with water, acidified to pH 6 with saturated NH_4Cl, and extracted with EtOAc. The combined organic layers were washed twice with water, 5% $Na_2S_2O_3$, water, and brine; dried with Na_2SO_4; and concentrated in vacuo. The crude material was purified by flash chromatography (hexane/EtOAc, 8:2) to yield **10** (542 mg, 1.54 mmol, 95%) as a colorless oil and a mixture of rotamers. R_f = 0.35 (hexane/EtOAc, 8:2). $[\alpha]_D^{20}$ = –49.0 (*c* = 1.2, $CHCl_3$). **Major rotamer:** ^1H NMR (400 MHz, $CDCl_3$): δ = 0.93 (d, $^3J_{12,11}$ = 6.6 Hz, 6 H, 12-H), 1.41 (s, 9 H, 15-H), 1.98 (m, 1 H, 11-H), 2.80 (s, 3 H, 16-H), 3.90 (s, 3 H, 1-H), 4.06–4.15 (m, 3 H, 9-H, 10-H), 7.06 (d, $^3J_{6,5}$ = 8.2 Hz, 1 H, 6-H), 7.32 (dd, $^3J_{5,6}$ = $^3J_{5,4}$ = 8.0 Hz, 1 H, 5-H), 7.53 (s, 1 H, 8-H), 7.62 (d, $^3J_{4,5}$ = 7.6 Hz, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.9 (q, C-12), 27.0 (d, C-11), 28.4 (q, C-15), 30.4 (q, C-16), 52.1 (q, C-1), 60.3 (d, C-10), 67.5 (t, C-9), 79.3 (s, C-14), 114.6 (d, C-8), 119.9 (d, C-6), 122.1 (d, C-4), 129.4 (d, C-5), 131.4 (s, C-3), 156.3 (s, C-13), 158.6 (s, C-7), 166.9 (s, C-2) ppm. **Minor rotamer:** ^1H NMR (400 MHz, $CDCl_3$): δ = 1.01 (d, $^3J_{12,11}$ = 6.4 Hz, 3 H, 12-H), 1.02 (d, $^3J_{12',11}$ = 6.4 Hz, 3 H, 12′-H), 1.46 (s, 9 H, 15-H), 2.09 (m, 1 H, 11-H), 2.81 (s, 3 H, 16-H), 3.90 (m, 1 H, 10-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.8 (q, C-12), 20.1 (q, C-12′), 27.8 (d, C-11), 61.6 (d, C-10), 68.3 (t, C-9), 79.5 (s, C-14), 114.9 (d, C-8), 120.1 (d, C-6), 122.2 (d, C-4), 129.4 (d, C-5), 156.4 (s, C-13), 158.8 (s, C-7) ppm. $C_{19}H_{29}NO_5$ (351.44): calcd. C 64.93, H 8.32, N 3.99; found C 64.90, H 8.09, N 4.22. HRMS (CI): calcd. for $C_{19}H_{30}NO_5$ $[M + H]^+$ 352.2124; found 352.2135.

Methyl 3-{(*R*)-3-[(2*S*,3*S*)-2-(Benzyloxycarbonylamino)-*N*,3-dimethylpentanamido]-4-methylpentyl}benzoate (11): At 0 °C, a solution of HCl in dioxane (4 M, 2.50 mL, 10.0 mmol) was added to compound **7** (357 mg, 1.02 mmol). The solvent was evaporated in vacuo after complete deprotection (2 h) and the hydrochloride salt was dried in vacuo. This salt was dissolved in dry dichloromethane (10 mL) together with *Z*-protected (*S*)-isoleucine (320 mg, 1.21 mmol) and 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP) (310 mg, 1.13 mmol) before diisopropylethylamine (0.52 mL, 3.06 mmol) was added dropwise at –10 °C. The cooling bath was removed after 20 min and the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with dichloromethane and washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, water, and brine; dried with Na_2SO_4; and concentrated in vacuo. The crude product was purified by flash chromatography (hexane/EtOAc, 7:3) to provide dipeptide **11** (472 mg, 0.95 mmol, 93% over 2 steps) as a colorless oil and a mixture of rotamers. R_f = 0.25 (hexane/EtOAc, 7:3). $[\alpha]_D^{21}$ = –15.6 (*c* = 1.4, $CHCl_3$). **Major rotamer:** ^1H NMR (400 MHz, $CDCl_3$): δ = 0.77 (d, $^3J_{13,12}$ = 6.6 Hz, 3 H, 13-H), 0.89 (t, $^3J_{19,18}$ = 7.4 Hz, 3 H, 19-H), 0.94 (d, $^3J_{13',12}$ = 6.5 Hz, 3 H, 13′-H), 1.01 (d, $^3J_{20,17}$ = 6.7 Hz, 3 H, 20-H), 1.15 (m, 1 H, 18-H_a), 1.52–1.72 (m, 3 H, 10-H_a, 12-H, 18-H_b), 1.80 (m, 1 H, 17-H), 1.94 (m, 1 H, 10-H_b), 2.50 (m, 2 H, 9-H), 2.98 (s, 3 H, 14-H), 3.90

(s, 3 H, 1-H), 4.34 (m, 1 H, 11-H), 4.58 (dd, $^3J_{16,NH}$ = 9.3, $^3J_{16,17}$ = 6.8 Hz, 1 H, 16-H), 5.09 (m, 2 H, 22-H), 5.51 (d, $^3J_{NH,16}$ = 9.4 Hz, 1 H, NH), 7.25–7.34 (m, 7 H, 5-H, 6-H, 24-H, 25-H, 26-H), 7.83 (s, 1 H, 8-H), 7.86 (m, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2 (q, C-19), 16.0 (q, C-20), 19.5 (q, C-13), 20.0 (q, C-13′), 23.8 (t, C-18), 29.2 (q, C-14), 30.2 (d, C-12), 31.4 (t, C-10), 32.7 (t, C-9), 37.5 (d, C-17), 52.0 (q, C-1), 55.8 (d, C-16), 59.3 (d, C-11), 66.7 (t, C-22), 127.2 (d, C-4), 127.8, 128.0, 128.4, 128.5 (4d, C-5, C-24, C-25, C-26), 129.1 (d, C-8), 130.3 (s, C-3), 132.9 (d, C-6), 136.4 (s, C-23), 142.2 (s, C-7), 156.4 (s, C-21), 167.1 (s, C-2), 173.1 (s, C-15) ppm. **Minor rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.04 (d, $^3J_{20,17}$ = 6.6 Hz, 3 H, 20-H), 1.87 (m, 1 H, 17-H), 2.01 (m, 1 H, 10-H), 2.35 (dt, $^2J_{9a,9b}$ = 13.2, $^3J_{9,10}$ = 4.3 Hz, 1 H, 9-H$_a$), 2.91 (s, 3 H, 14-H), 3.60 (m, 1 H, 11-H), 3.89 (s, 3 H, 1 H), 4.67 (dd, $^3J_{16,NH}$ = 9.7, $^3J_{16,17}$ = 6.4 Hz, 1 H, 16-H), 7.80 (s, 1 H, 8-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.3 (q, C-19), 16.1 (q, C-20), 20.3 (q, C-13), 20.5 (q, C-13′), 23.6 (t, C-18), 27.2 (q, C-14), 38.0 (d, C-17), 55.2 (d, C-16), 63.1 (d, C-11), 66.8 (t, C-22), 132.9 (d, C-6), 136.3 (s, C-23), 141.9 (s, C-7), 156.1 (s, C-21), 167.1 (s, C-2), 172.8 (s, C-15) ppm. HRMS (CI): calcd. for $C_{29}H_{41}N_2O_5$ [M + H]$^+$ 497.3015; found 497.3005.

Methyl 3-{(*S*)-2-[(2*S*,3*S*)-2-(Benzyloxycarbonylamino)-*N*,3-dimethylpentanamido]-3-methylbutoxy}benzoate (12): According to the preparation of dipeptide **11**, compound **12** was obtained by deprotection of **10** (512 mg, 1.48 mmol) in HCl/dioxane (4 M, 3.80 mL, 15.0 mmol). Subsequent peptide coupling with Z-protected L-isoleucine (429 mg, 1.62 mmol), BEP (448 mg, 1.64 mmol), and diisopropyl ethylamine (0.75 mL, 4.41 mmol) in dry dichloromethane (15 mL) gave rise to **12** (693 mg, 1.39 mmol, over 2 steps) as a colorless oil and a mixture of rotamers. R_f = 0.21 (hexane/EtOAc, 7:3). $[\alpha]_D^{20}$ = –38.0 (c = 1.3, $CHCl_3$). **Major rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.84–0.90 (m, 6 H, 12-H, 18-H), 0.98 (d, $^3J_{19,16}$ = 6.7 Hz, 3 H, 19-H), 1.01 (d, $^3J_{12',11}$ = 6.9 Hz, 3 H, 12′-H), 1.15 (m, 1 H, 17-H$_a$), 1.60 (m, 1 H, 17-H$_b$), 1.77 (m, 1 H, 16-H), 2.03 (m, 1 H, 11-H), 3.06 (s, 3 H, 13-H), 3.90 (s, 3 H, 1-H), 4.10 (m, 2 H, 9-H), 4.56 (dd, $^3J_{15,NH}$ = 9.0, $^3J_{15,16}$ = 7.5 Hz, 1 H, 15-H), 4.65 (br. s, 1 H, 10-H), 5.09 (s, 2 H, 21-H), 5.51 (d, $^3J_{NH,15}$ = 9.3 Hz, 1 H, NH), 7.03 (dd, $^3J_{6,5}$ = 8.3, $^4J_{6,4}$ = 2.0 Hz, 1 H, 6-H), 7.27–7.34 (m, 6 H, 5-H, 23-H, 24-H, 25-H), 7.51 (s, 1 H, 8-H), 7.63 (d, $^3J_{4,5}$ = 7.8 Hz, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2 (q, C-18), 15.5 (q, C-19), 19.5 (q, C-12), 19.8 (q, C-12′), 24.0 (t, C-17), 27.0 (d, C-11), 30.5 (q, C-13), 37.7 (d, C-16), 52.1 (q, C-1), 55.5 (d, C-15), 58.3 (d, C-10), 66.7 (d, C-21), 67.0 (t, C-9), 114.5 (d, C-8), 119.7 (d, C-6), 122.3 (d, C-4), 127.8, 127.9, 128.4 (3d, C-23, C-24, C-25), 129.4 (d, C-5), 131.5 (s, C-3), 136.4 (s, C-22), 156.4 (s, C-20), 158.5 (s, C-7), 166.7 (s, C-2), 173.2 (s, C-14) ppm. **Minor rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.01 (d, $^3J_{12,11}$ = 6.9 Hz, 3 H, 12-H), 1.12 (d, $^3J_{12',11}$ = 6.6 Hz, 3 H, 12′-H), 1.86 (m, 1 H, 16-H), 2.82 (s, 3 H, 13-H), 3.87 (s, 3 H, 1-H), 3.94–4.01 (m, 3 H, 9-H, 10-H), 4.78 (dd, $^3J_{15,NH}$ = 9.4, $^3J_{15,16}$ = 6.3 Hz, 1 H, 15-H), 5.00 (d, $^2J_{21a,21b}$ = 12.3 Hz, 1 H, 21-H$_a$), 5.60 (d, $^3J_{NH,15}$ = 9.5 Hz, 1 H, NH), 7.00 (m, 1 H, 6-H), 7.20 (dd, $^3J_{5,6}$ = $^3J_{5,4}$ = 8.0 Hz, 1 H, 5-H), 7.48 (s, 1 H, 8-H), 7.61 (m, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.4 (q, C-18), 16.1 (q, C-19), 20.2 (q, C-12), 23.6 (t, C-17), 37.9 (d, C-16), 37.9 (d, C-16), 52.1 (q, C-1), 62.1 (d, C-10), 66.6 (d, C-21), 114.0 (d, C-8), 120.3 (d, C-6), 122.3 (d, C-4), 127.9, 128.0, 128.4 (3d, C-23, C-24, C-25), 129.4 (d, C-5), 131.2 (s, C-3), 156.2 (s, C-20), 158.3 (s, C-7), 173.4 (s, C-14) ppm. HRMS (CI): calcd. for $C_{28}H_{39}N_2O_6$ [M + H]$^+$ 499.2808; found 499.2804.

Methyl 3-((*R*)-3-{(2*S*,3*S*)-*N*,3-Dimethyl-2-[(*R*)-1-(3-phenylpropanoyl)piperidine-2-carboxamido]pentanamido}-4-methylpentyl)benzoate (13): A solution of dipeptide **11** (230 mg, 0.38 mmol) in MeOH (7 mL) was stirred under hydrogen in the presence of 10% Pd/C (24 mg) until the deprotection was complete (1 h). After filtration through Celite, the solvent was removed in vacuo and the free amine was dried in vacuo. Isobutyl chloroformate (63 μL, 0.47 mmol) was added to a solution of *Z*-protected (*R*)-pipecolic acid (125 mg, 0.47 mmol) and *N*-methylmorpholine (155 μL, 1.41 mmol) in dry THF (7 mL) at –20 °C. After 20 min, the free amine dissolved in dry THF (6 mL) was added and the mixture was warmed to room temperature overnight. Water was added and the mixture was extracted thrice with EtOAc. The combined organic layers were washed with 1 M aqueous HCl, water, saturated aqueous $NaHCO_3$, water, and brine; dried with Na_2SO_4; and concentrated in vacuo. The crude product was purified by flash chromatography (hexane/EtOAc, 6:4) to yield tripeptide **13** (680 mg, 1.12 mmol, 89% over 2 steps) as a white solid and a mixture of rotamers; m.p. 38–40 °C. R_f = 0.22 (hexane/EtOAc, 6:4). $[\alpha]_D^{21}$ = –7.2 (c = 1.0, $CHCl_3$). **Major rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.76 (d, $^3J_{13,12}$ = 6.6 Hz, 3 H, 13-H), 0.85 (br. s, 3 H, 19-H), 0.94 (d, $^3J_{13',12}$ = 6.5 Hz, 3 H, 13′-H), 0.97 (br. s, 3 H, 20-H), 1.09 (m, 1 H, 18-H$_a$), 1.25–1.71 (m, 8 H, 10-H$_a$, 12-H, 18-H$_b$, 23-H$_a$, 24-H, 25-H), 1.81 (m, 1 H, 17-H), 1.95 (m, 1 H, 10-H$_b$), 2.31 (m, 2 H, 23-H$_b$), 2.49 (m, 2 H, 9-H), 2.89 (br. s, 1 H, 26-H$_a$), 3.01 (s, 3 H, 14-H), 3.90 (s, 3 H, 1-H), 4.08 (br. s, 1 H, 26-H$_b$), 3.34 (m, 1 H, 11-H), 4.84 (dd, $^3J_{16,NH}$ = 8.7, $^3J_{16,17}$ = 7.9 Hz, 1 H, 16-H), 4.91 (br. s, 1 H, 22-H), 5.17 (m, 2 H, 28-H), 6.70 (br. s, 1 H, NH), 7.27–7.40 (m, 7 H, 5-H, 6-H, 30-H, 31-H, 32-H), 7.82 (s, 1 H, 8-H), 7.87 (m, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.1 (q, C-19), 15.9 (q, C-20), 19.6 (q, C-13), 20.0 (q, C′-13), 20.4 (t, C-25), 24.1 (t, C-18), 24.8 (t, C-24), 25.6 (t, C-23), 29.2 (q, C-14), 30.2 (d, C-12), 31.4 (t, C-10), 32.8 (t, C-9), 37.2 (d, C-17), 42.3 (t, C-26), 52.1 (q, C-1), 53.7 (d, C-16), 55.0 (d, C-22), 59.3 (d, C-11), 67.6 (t, C-28), 127.3 (d, C-4), 127.8, 128.0, 128.5 (4d, C-5, C-30, C-31, C-32), 129.1 (d, C-8), 130.3 (s, C-3), 132.9 (d, C-6), 136.4 (s, C-29), 142.2 (s, C-7), 156.4 (s, C-21), 167.2 (s, C-2), 170.5 (s, C-21), 173.1 (s, C-15) ppm. **Minor rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.04 (d, $^3J_{20,17}$ = 6.5 Hz, 3 H, 20-H), 2.01 (m, 1 H, 10-H$_b$), 2.26 (m, 1 H, 23-H$_b$), 2.64 (m, 2 H, 9-H), 2.81 (s, 3 H, 14-H), 2.63 (m, 1 H, 11-H), 3.88 (s, 3 H, 1-H), 4.20 (br. s, 1 H, 26-H$_b$), 4.34 (m, 1 H, 11-H), 5.05–5.07 (m, 2 H, 16-H, 28-H$_a$), 6.58 (br. s, 1 H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.3 (q, C-19), 16.2 (q, C-20), 20.3 (q, C-13), 20.6 (t, C-25), 23.6 (t, C-18), 24.6 (t, C-24), 26.1 (t, C-23), 27.3 (q, C-14), 31.3 (d, C-12), 32.2 (t, C-10), 33.2 (t, C-9), 38.2 (d, C-17), 52.0 (q, C-1), 53.0 (d, C-16), 55.4 (d, C-22), 63.3 (d, C-11), 67.5 (t, C-28), 155.5 (s, C-21) ppm. HRMS (CI): calcd. for $C_{35}H_{50}N_3O_6$ [M + H]$^+$ 608.3699; found 608.3729.

Methyl 3-((*S*)-2-{(2*S*,3*S*)-*N*,3-Dimethyl-2-[(*R*)-1-(3-phenylpropanoyl)piperidine-2-carboxamido]pentanamido}-3-methylbutoxy)benzoate (14): According to the preparation of tripeptide **13**, compound **14** was obtained by deprotection of **12** (629 mg, 1.26 mmol) in MeOH (7 mL). Subsequent peptide coupling with Z-protected (*R*)-pipecolic acid (366 mg, 1.39 mmol), isobutyl chloroformate (185 μL, 1.39 mmol), and *N*-methylmorpholine (155 μL, 1.41 mmol) in dry THF (7 mL) gave rise to **14** (680 mg, 1.12 mmol, 89% over 2 steps) as a white solid and a mixture of rotamers; m.p. 38–40 °C. R_f = 0.13 (hexane/EtOAc, 7:3). $[\alpha]_D^{20}$ = –7.2 (c = 1.0, $CHCl_3$). **Major rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.79–0.88 (m, 6 H, 12-H, 18-H), 0.95 (d, $^3J_{19,16}$ = 5.5 Hz, 3 H, 19-H), 1.04 (d, $^3J_{12,11}$ = 6.5 Hz, 3 H, 12-H), 1.09 (m, 1 H, 17-H$_a$), 1.26–1.44 (m, 2 H, 23-H$_a$, 24-H$_a$), 1.45–1.66 (m, 4 H, 17-H$_b$, 22-H$_a$, 23-H$_b$, 24-H$_b$), 1.80 (m, 1 H, 16-H), 2.00 (m, 1 H, 11-H), 2.30 (m, 1 H, 22-H$_b$), 2.88 (m, 1 H, 25-H$_a$), 3.09 (s, 3 H, 13-H), 3.91 (s, 3 H, 1-H), 4.02–4.12 (m, 3 H, 9-H, 25-H$_b$), 4.67 (br. s, 1 H, 10-H), 4.80

(dd, $^3J_{15,NH} = {}^3J_{15,16}$ = 8.5 Hz, 1 H, 15-H), 4.91 (br. s, 1 H, 21-H), 5.17 (m, 2 H, 27-H), 6.59 (br. s, 1 H, NH), 7.02 (dd, $^3J_{6,5}$ = 8.1, $^4J_{6,4}$ = 1.6 Hz, 1 H, 6-H), 7.25–7.40 (m, 6 H, 5-H, 29-H, 30-H, 31-H), 7.50 (s, 1 H, 8-H), 7.63 (d, $^3J_{4,6}$ = 7.8 Hz, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.0 (q, C-18), 15.4 (q, C-19), 19.5 (q, C-12), 19.8 (q, C-12′), 20.4 (t, C-23), 24.4 (t, C-17), 24.8 (t, C-24), 25.7 (t, C-22), 26.7 (d, C-11), 27.3 (q, C-13), 37.2 (d, C-16), 42.3 (t, C-25), 52.1 (q, C-1), 53.5 (d, C-15), 55.0 (C-21), 58.3, (C-10), 66.9 (t, C-9), 67.6 (t, C-27), 114.4 (d, C-8), 119.7 (d, C-6), 122.3 (d, C-4), 127.8, 128.0, 128.5 (3d, C-29, C-30, C-31), 129.4 (d, C-5), 131.5 (s, C-3), 136.4 (s, C-28), 156.4 (s, C-26), 158.5 (s, C-7), 166.8 (s, C-2), 170.6 (s, C-20), 173.3 (s, C-14) ppm. **Minor rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.01 (d, $^3J_{12,11}$ = 6.7 Hz, 3 H, 12-H), 1.11 (d, $^3J_{12',11}$ = 6.5 Hz, 3 H, 12′-H), 2.11 (m, 1 H, 11-H), 2.36 (m, 1 H, 22-H$_b$), 2.85 (s, 3 H, 13-H), 2.98 (m, 1 H, 25-H$_a$), 3.89 (s, 3 H, 1-H), 4.20 (br. s, 1 H, 25-H$_b$), 6.77 (d, $^3J_{NH,13}$ = 9.2 Hz, 1 H, NH), 7.10 (m, 1 H, 6-H), 7.48 (s, 1 H, 8-H), 7.61 (m, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.4 (q, C-18), 16.1 (q, C-19), 20.1 (q, C-12), 20.3 (q, C-12′), 26.1 (t, C-22), 30.5 (q, C-13), 37.7 (d, C-16), 52.1 (q, C-1), 55.4 (C-21), 62.1 (d, C-10), 67.2 (t, C-9), 67.5 (t, C-27), 113.0 (d, C-8), 120.7 (d, C-6), 122.6 (d, C-4), 131.3 (s, C-3), 158.4 (s, C-7), 170.1 (s, C-20) ppm. HRMS (CI): calcd. for $C_{34}H_{48}N_3O_7$ $[M + H]^+$ 610.3492; found 610.3454.

Benzyl (*R*)-2-{(2*S*,3*S*)-1-[((*R*)-1-{3-[(2*R*,4*S*)-5-Methoxy-4-methyl-5-oxo-1-phenylpentan-2-ylcarbamoyl]phenyl}-4-methylpentan-3-yl)methylamino]-3-methyl-1-oxopentan-2-ylcarbamoyl}piperidine-1-carboxylate (15): A mixture of methyl ester **13** (180 mg, 0.30 mmol) and 1 M aqueous NaOH (0.45 mL, 0.45 mmol) in MeOH (3 mL) was stirred at 60 °C until complete saponification occurred (3 h). The solvent was evaporated in vacuo and the residue was dissolved in water, acidified to pH 1 with 1 M aqueous $KHSO_4$, and extracted thrice with EtOAc. The combined organic layers were dried with Na_2SO_4 and the solvent was evaporated in vacuo to give the crude acid as a white foam. Isobutyl chloroformate (39 μL, 0.30 mmol) was added to a solution of the crude acid and *N*-methylmorpholine (78 μL, 0.71 mmol) in dry THF (6 mL) at –20 °C. After 20 min Tup-OMe·HCl (78 mg, 0.30 mmol) was added and the mixture was warmed to room temperature overnight. Water was added and the mixture was extracted thrice with EtOAc. The combined organic layers were washed with 1 M aqueous HCl, water, saturated aqueous $NaHCO_3$, water, and brine; dried with Na_2SO_4; and concentrated in vacuo. The crude product was purified by flash chromatography (hexane/EtOAc, 1:1) to yield tetrapeptide **15** (165 mg, 0.21 mmol, 73% over 2 steps) as a white solid and a mixture of rotamers; m.p. 59–61 °C. R_f = 0.23 (hexane/EtOAc, 1:1). $[\alpha]_D^{20}$ = +23.1 (*c* = 0.9, $CHCl_3$). **Major rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.74 (d, $^3J_{22,21}$ = 6.6 Hz, 3 H, 22-H), 0.84 (m, 3 H, 28-H), 0.89 (d, $^3J_{22,21}$ = 6.5 Hz, 3 H, 22′-H), 0.93–1.03 (m, 4 H, 27-H$_a$, 29-H), 1.16 (d, $^3J_{3,2}$ = 7.1 Hz, 3 H, 3-H), 1.28–1.48 (m, 2 H, 33-H$_a$, 34-H$_a$), 1.48–1.76 (m, 7 H, 4-H$_a$, 19-H$_a$, 21-H, 27-H$_b$, 32-H$_a$, 33-H$_b$, 34-H$_b$), 1.76–1.92 (m, 2 H, 19-H$_b$, 26-H), 1.98 (m, 1 H, 4-H$_b$), 2.27 (m, 1 H, 32-H$_b$), 2.50 (m, 1 H, 18-H), 2.64 (m, 1 H, 2-H), 2.88 (dd, $^2J_{6a,6b}$ = 13.6, $^3J_{6a,5}$ = 6.8 Hz, 1 H, 6-H$_a$), 2.82–3.08 (m, 3 H, 6-H$_b$, 23-H, 35-H$_a$), 3.60 (s, 3 H, 42-H), 4.06 (m, 1 H, 35-H$_b$), 4.31 (m, 1 H, 20-H), 4.41 (m, 1 H, 5-H), 4.76–4.91 (m, 2 H, 25-H, 31-H), 5.11 (d, $^2J_{37a,37b}$ = 12.5 Hz, 1 H, 37-H$_a$), 5.18 (d, $^3J_{37b,37a}$ = 12.5 Hz, 1 H, 37-H$_b$), 6.41 (br. s, 1 H, NH), 6.78 (br. s, 1 H, NH), 7.18–7.36 (m, 12 H, 8-H, 9-H, 10-H, 14-H, 15-H, 39-H, 40-H, 41-H), 7.46 (m, 1 H, 13-H), 7.58 (m, 1 H, 17-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.0 (q, C-28), 15.8 (q, C-29), 17.3 (q, C-3), 19.5 (q, C-22), 19.9 (q, C-22′), 20.2 (t, C-33), 24.2 (t, C-27), 24.6 (t, C-34), 25.9 (t, C-32), 29.4 (q, C-23), 30.2 (d, C-21), 31.3 (t, C-19), 32.6 (t, C-18), 36.4 (d, C-2), 37.0, 37.1 (d, t, C-4, C-26), 40.7 (t, C-6), 42.2 (t, C-35), 49.0 (d, C-5), 51.8 (q, C-42), 53.8 (d, C-25), 54.9 (d, C-31), 58.9 (d, C-20), 67.7 (t, C-37), 124.1 (d, C-13), 126.5, 127.8, 127.7, 128.0, 128.3, 128.4, 128.6, 129.4 (s, 8d, C-8, C-9, C-10, C-12, C-14, C-17, C-39, C-40, C-41), 131.4 (s, C-15), 136.2 (s, C-38), 137.7 (s, C-7), 142.1 (s, C-16), 155.6 (s, C-36), 167.0 (s, C-11), 170.4 (s, C-30), 173.5 (s, C-24), 177.0 (s, C-1) ppm. **Selected minor rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.02 (d, $^3J_{22,11}$ = 6.5 Hz, 3 H, 22-H), 2.78 (s, 3 H, 23-H), 3.57 (m, 1 H, 20-H), 4.20 (m, 1 H, 35-H$_b$), 4.97–5.05 (m, 3 H, 25-H, 37-H), 7.50–7.52 (m, 2 H, 13-H, 17-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2 (q, C-28), 16.2 (q, C-29), 17.5 (q, C-3), 20.3 (q, C-22), 20.5 (q, C-22′), 27.4 (q, C-23), 31.3 (d, C-21), 32.0 (t, C-19), 32.9 (t, C-18), 63.2 (d, C-20), 67.5 (t, C-37), 137.9 (s, C-7), 141.7 (s, C-16), 155.5 (s, C-36), 176.9 (s, C-1) ppm. HRMS (CI): calcd. for $C_{47}H_{66}N_4O_7$ $[M + 2H]^{2+}$ 798.4931; found 798.4973.

Benzyl (*R*)-2-{(2*S*,3*S*)-1-[((*S*)-1-{3-[(2*R*,4*S*)-5-Methoxy-4-methyl-5-oxo-1-phenylpentan-2-ylcarbamoyl]phenoxy}-3-methylbutan-2-yl)methylamino]-3-methyl-1-oxopentan-2-ylcarbamoyl}piperidine-1-carboxylate (16): According to the preparation of tetrapeptide **15**, compound **14** (610 mg, 1.00 mmol) was subjected to saponification with 1 M NaOH (1.25 mL, 1.5 mmol) in MeOH (10 mL). Subsequent peptide coupling of the crude acid (395 mg, 0.66 mmol) with Tup-OMe·HCl (190 mg, 0.74 mmol), isobutyl chloroformate (0.92 mL, 0.69 mmol), and *N*-methylmorpholine (175 μL, 1.59 mmol) in dry THF (6 mL) gave rise to **16** (366 mg, 0.46 mmol, 70%) as a white solid and a mixture of rotamers; m.p. 58–60 °C. R_f = 0.20 (hexane/EtOAc, 1:1). $[\alpha]_D^{20}$ = +1.2 (*c* = 1.4, $CHCl_3$). **Major rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.78–0.88 (m, 6 H, 21-H, 27-H), 0.94 (d, $^3J_{28,25}$ = 6.5 Hz, 3 H, 28-H), 1.04 (d, $^3J_{21,20}$ = 6.5 Hz, 3 H, 21-H), 1.10 (m, 1 H, 26-H$_a$), 1.18 (d, $^3J_{3,2}$ = 7.15 Hz, 3 H, 3-H), 1.32–1.45 (m, 2 H, 32-H$_a$, 32-H$_b$), 1.45–1.65 (m, 4 H, 26-H$_a$, 31-H$_a$, 32-H$_b$, 33-H$_b$), 1.69 (ddd, $^2J_{4a,4b}$ = 14.2, $^3J_{4,2}$ = 9.7, $^3J_{4,5}$ = 4.2 Hz, 1 H, 4-H$_a$), 1.81 (m, 1 H, 25-H$_a$), 1.93–2.05 (m, 2 H, 4-H$_b$, 20-H), 2.28 (m, 1 H, 31-H$_b$), 2.66 (m, 1 H, 2-H), 2.89 (dd, $^2J_{6a,6b}$ = 13.8, $^3J_{6a,5}$ = 6.8 Hz, 1 H, 6-H$_a$), 2.93 (m, 1 H, 34-H$_a$), 2.98 (dd, $^2J_{6b,6a}$ = 13.6, $^3J_{6b,5}$ = 5.9 Hz, 1 H, 6-H$_b$), 3.08 (s, 3 H, 22-H), 3.63 (s, 3 H, 41-H), 4.00–4.25 (m, 3 H, 18-H, 34-H$_b$), 4.41 (m, 1 H, 5-H), 4.69 (m, 1 H, 19-H), 4.78 (m, 1 H, 24-H), 4.87 (m, 1 H, 30-H), 5.15 (d, $^2J_{36a,36b}$ = 12.6 Hz, 1 H, 36-H$_a$), 5.20 (d, $^2J_{36b,36a}$ = 12.5 Hz, 1 H, 36-H$_b$), 6.23 (br. s, 1 H, NH), 6.79 (br. s, 1 H, NH), 6.94 (d, $^3J_{15,14}$ = 7.8 Hz, 1 H, 15-H), 7.16 (d, $^3J_{13,14}$ = 7.7 Hz, 1 H, 13-H), 7.19–7.26 (m, 3 H, 8-H, 10-H), 7.27–7.38 (m, 9 H, 9-H, 14-H, 17-H, 38-H, 39-H, 40-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.0 (q, C-27), 15.4 (q, C-28), 17.3 (q, C-3), 19.4 (q, C-21), 19.7 (q, C-21′), 20.3 (t, C-32), 24.4 (t, C-26), 24.6 (t, C-33), 25.8 (t, C-31), 26.8 (d, C-20), 30.4 (d, C-20), 30.4 (d, C-2), 37.0 (t, C-4), 37.1 (d, C-25), 40.7 (t, C-6), 42.3 (t, C-34), 49.0 (d, C-5), 51.8 (q, C-41), 53.7 (d, C-24), 54.9 (d, C-30), 58.2 (d, C-19), 66.5 (t, C-18), 67.7 (t, C-36), 112.6 (d, C-17), 118.4 (d, C-15), 118.7 (d, C-13), 126.5, 127.8, 128.0, 128.4, 128.5, 129.4, 129.5 (7d, C-8, C-9, C-10, C-14, C-38, C-39, C-40), 136.0 (s, C-12), 136.3 (s, C-37), 137.6 (s, C-7), 156.6 (s, C-35), 158.7 (s, C-16), 166.5 (s, C-11), 170.2 (s, C-29), 173.5 (s, C-23), 177.0 (s, C-1) ppm. **Minor rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.01 (d, $^3J_{21,20}$ = 6.7 Hz, 3 H, 21-H), 1.11 (d, $^3J_{21,20}$ = 6.4 Hz, 3 H, 21-H), 2.09 (m, 1 H, 20-H), 2.84 (s, 3 H, 22-H), 3.61 (s, 3 H, 41-H), 3.89 (m, 1 H, 19-H), 6.55 (br. s, 1 H, NH), 7.01 (m, 1 H, 15-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.4 (q, C-27), 16.1 (q, C-28), 17.4 (q, C-3), 20.0 (q, C-21), 26.1 (t, C-31), 36.4 (d, C-20), 40.8 (t, C-6), 42.0 (t, C-34), 49.1 (d, C-5), 51.7 (q, C-41), 55.4 (d, C-30), 62.3 (d, C-19), 67.5 (t, C-36), 137.8 (s, C-7), 155.4 (s, C-35), 158.6 (s, C-16), 166.5 (s, C-11), 173.0 (s, C-23), 176.9 (s, C-1) ppm. HRMS (CI): calcd. for $C_{39}H_{55}N_4O_7$ $[M - C_7H_7O]^+$ 691.4071; found 691.4100.

Methyl (2*S*,4*R*)-4-[3-((*R*)-3-{(2*S*,3*S*)-*N*,3-Dimethyl-2-[(*R*)-1-methylpiperidine-2-carboxamido]pentanamido}-4-methylpentyl)benzamido]-2-methyl-5-phenylpentanoate (17): A solution of tetrapeptide **15** (253 mg, 0.32 mmol) in MeOH (4 mL) was stirred under hydrogen in the presence of 10% Pd/C (27 mg) until deprotection was complete (3 h). After filtration through Celite, the solvent was removed in vacuo and the free amine was dried in vacuo. This crude amine was dissolved in dry MeOH (4 mL) and paraformaldehyde (29 mg, 0.32 mmol) was added. After the solution had been stirred for 3 h at room temperature, sodium cyanoborohydride (22 mg, 0.36 mmol) was added and the reaction mixture was stirred for further 16 h. The methanol was removed, the residue was diluted with saturated aqueous $NaHCO_3$, and the solution was extracted thrice with dichloromethane. The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography (dichloromethane/MeOH, 97:3, 95:5) to yield the *N*-methylated tetrapeptide **17** (168 mg, 0.25 mmol, 78% over 2 steps) as a white solid and a mixture of rotamers; m.p. 45–47 °C. R_f = 0.26 (dichloromethane/MeOH, 95:5). $[\alpha]_D^{21}$ = +35.2 (*c* = 1.2, $CHCl_3$). **Major rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.73 (d, $^3J_{22,21}$ = 6.6 Hz, 3 H, 22-H), 0.86 (d, $^3J_{22',21}$ = 6.4 Hz, 3 H, 22′-H), 0.89 (t, $^3J_{28,27}$ = 7.4 Hz, 3 H, 28-H), 0.99 (d, $^3J_{29,26}$ = 6.8 Hz, 3 H, 29-H), 1.16 (d, $^3J_{3,2}$ = 7.2 Hz, 3 H, 3-H), 1.15–1.25 (m, 2 H, 27-H_a, 33-H_a), 1.36 (m, 1 H, 32-H_a), 1.45–1.74 (m, 7 H, 4-H_a, 19-H_a, 21-H, 27-H_b, 33-H_b, 34-H), 1.79–1.92 (m, 3 H, 19-H_b, 26-H, 32-H_b), 1.97–2.04 (m, 2 H, 4-H_b, 35-H_a), 2.24 (s, 3 H, 36-H), 2.40–2.50 (m, 2 H, 18-H_a, 31-H), 2.55 (m, 1 H, 18-H_b), 2.65 (m, 1 H, 2-H), 2.84–2.93 (m, 2 H, 6-H_a, 35-H_b), 2.95–3.04 (m, 4 H, 6-H_b, 23-H), 3.60 (s, 3 H, 37-H), 4.28 (br. s, 1 H, 20-H), 4.41 (m, 1 H, 5-H), 4.81 (dd, $^3J_{25,26}$ = $^3J_{25,NH}$ = 8.9 Hz, 1 H, 25-H), 6.60 (d, $^3J_{NH,5}$ = 8.2 Hz, 1 H, NH), 7.06 (d, $^3J_{NH,25}$ = 9.5 Hz, 1 H, NH), 7.18–7.33 (m, 7 H, 8-H, 9-H, 10-H, 14-H, 15-H), 7.51–7.56 (m, 13-H, 17-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.9 (q, C-28), 15.9 (q, C-29), 17.6 (q, C-3), 19.6 (q, C-22), 20.0 (q, C-22′), 23.2 (t, C-33), 24.7 (t, C-27), 25.1 (t, C-34), 29.5 (q, C-23), 30.2 (d, C-21), 30.4 (t, C-32), 31.4 (t, C-19), 32.6 (t, C-18), 36.4 (d, C-2), 37.2, 37.3 (d, t, C-4, C-26), 41.2 (t, C-6), 44.9 (q, C-36), 49.1 (d, C-5), 51.7 (q, C-37), 53.0 (d, C-25), 55.4 (t, C-35), 68.4 (d, C-20), 69.6 (d, C-31), 124.6 (d, C-13), 126.4 (d, C-10), 127.1 (d, C-17), 128.6 (d, C-14), 128.4, 129.4 (2d, C-8, C-9), 131.2 (d, C-15), 134.5 (s, C-12), 137.9 (s, C-7), 142.0 (s, C-16), 166.9 (s, C-11), 173.3 (s, C-30), 174.2 (s, C-24), 176.9 (s, C-1) ppm. **Minor rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.91–0.95 (m, 6 H, 22-H, 29-H), 2.22 (s, 3 H, 36-H), 2.79 (s, 3 H, 23-H), 3.56 (m, 1 H, 20-H), 3.61 (s, 3 H, 37-H), 5.01 (dd, $^3J_{25,NH}$ = 9.7 Hz, $^3J_{25,26}$ = 5.6 Hz, 1 H, 25-H), 6.53 (d, $^3J_{NH,5}$ = 8.4 Hz, 1 H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.4 (q, C-28), 16.5 (q, C-29), 20.3 (q, C-22), 20.6 (q, C-22′), 23.9 (t, C-33), 31.9 (t, C-19), 40.9 (t, C-6), 49.1 (q, C-36), 49.0 (d, C-5), 69.8 (d, C-31) ppm. $C_{40}H_{60}N_4O_5$ (676.93): calcd. C 70.97, H 8.93, N 8.28; found C 70.10, H 8.67, N 7.92. HRMS (CI): calcd. for $C_{40}H_{61}N_4O_5$ $[M + H]^+$ 677.4643; found 677.4634.

Methyl (2*S*,4*R*)-4-[3-((*S*)-2-{(2*S*,3*S*)-*N*,3-Dimethyl-2-[(*R*)-1-methylpiperidine-2-carboxamido]pentanamido}-3-methylbutoxy)benzamido]-2-methyl-5-phenylpentanoate (18): According to the preparation of *N*-methylated tetrapeptide **17**, compound **18** was obtained by deprotection of **16** (282 mg, 0.35 mmol) in MeOH (4 mL). Subsequent reductive methylation with paraformaldehyde (31 mg, 0.34 mmol) and sodium cyanoborohydride (24 mg, 0.38 mmol) in MeOH (4 mL) gave rise to **18** (177 mg, 0.26 mmol, 76%) as a white solid and a mixture of rotamers; m.p. 56–58 °C. R_f = 0.29 (dichloromethane/MeOH, 9:1). $[\alpha]_D^{20}$ = +6.8 (*c* = 1.4, $CHCl_3$). **Major rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.84 (d, $^3J_{21,20}$ = 6.6 Hz, 3 H, 21-H), 0.88 (t, $^3J_{27,26}$ = 7.4 Hz, 3 H, 27-H), 0.96 (d, $^3J_{28,25}$ = 6.7 Hz, 3 H, 28-H), 1.02 (d, $^3J_{21,20}$ = 6.3 Hz, 3 H, 21-H), 1.17 (d, $^3J_{3,2}$ = 7.2 Hz, 3 H, 3-H), 1.12–1.25 (m, 2 H, 26-H_a, 32-H_a), 1.36 (m, 1 H, 31-H_a), 1.42–1.72 (m, 5 H, 4-H_a, 26-H_b, 32-H_b, 33-H), 1.77 (m, 1 H, 31-H_b), 1.87 (m, 1 H, 25-H), 1.93–2.04 (m, 3 H, 4-H_b, 20-H, 34-H_a), 2.21 (s, 3 H, 35-H), 2.45 (dd, $^3J_{30,31a}$ = 10.7, $^3J_{30,31b}$ = 2.1 Hz, 1 H, 30-H), 2.65 (m, 1 H, 2-H), 2.86–2.91 (m, 2 H, 6-H_a, 34-H_b), 2.97 (dd, $^2J_{6b,6a}$ = 13.7, $^3J_{6b,5}$ = 5.9 Hz, 1 H, 6-H_b), 3.08 (s, 3 H, 22-H), 3.62 (s, 3 H, 36-H), 4.08 (m, 2 H, 18-H), 4.40 (m, 1 H, 5-H), 4.65 (br. s, 1 H, 19-H), 4.74 (dd, $^3J_{24,NH}$ = $^3J_{24,25}$ = 9.0 Hz, 1 H, 24-H), 6.21 (d, $^3J_{NH,5}$ = 7.3 Hz, 1 H, NH), 6.94 (dd, $^3J_{15,14}$ = 8.2, $^4J_{15,13}$ = 2.2 Hz, 1 H, 15-H), 7.01 (d, $^3J_{NH,24}$ = 9.3 Hz, 1 H, NH), 7.15–7.35 (m, 8 H, 8-H, 9-H, 10-H, 13-H, 14-H, 17-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.9 (q, C-27), 15.5 (q, C-28), 17.3 (q, C-3), 19.5 (q, C-21), 19.8 (q, C-21′), 23.2 (t, C-34), 24.8 (t, C-26), 25.1 (t, C-33), 26.9 (d, C-20), 30.3 (t, C-31), 30.7 (q, C-22), 36.4 (d, C-2), 37.0 (t, C-4), 37.2 (d, C-25), 40.7 (t, C-6), 44.8 (q, C-35), 48.9 (d, C-59), 51.8 (q, C-36), 52.8 (d, C-24), 55.4 (t, C-34), 58.1 (d, C-19), 67.1 (t, C-18), 69.7 (d, C-30), 112.5 (d, C-17), 118.3 (d, C-15), 118.7 (d, C-13), 126.5 (d, C-10), 128.4, 129.4 (2d, C-8, C-9), 129.5 (d, C-14), 136.1 (s, C-12), 137.6 (s, C-7), 158.8 (s, C-16), 166.5 (s, C-11), 173.2, 174.4 (2s, C-7, C-13), 177.0 (s, C-1) ppm. **Minor rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.10 (d, $^3J_{21,20}$ = 6.5 Hz, 3 H, 21-H), 1.14 (d, $^3J_{3,2}$ = 7.3 Hz, 3 H, 3-H), 2.05 (m, 1 H, 20-H), 2.25 (s, 3 H, 35-H), 2.45 (m, 1 H, 30-H), 2.78 (dd, $^2J_{6a,6b}$ = 13.5, $^3J_{6a,5}$ = 7.7 Hz, 1 H, 6-H_a), 2.84 (s, 3 H, 22-H), 3.03 (dd, $^2J_{6b,6a}$ = 13.8, $^3J_{6b,5}$ = 5.7 Hz, 1 H, 6-H_b), 3.58 (s, 3 H, 36-H), 3.85 (m, 1 H, 19-H), 5.22 (dd, $^3J_{24,NH}$ = 9.5, $^3J_{24,25}$ = 4.4 Hz, 1 H, 24-H), 6.75 (d, $^3J_{NH,5}$ = 8.4 Hz, 1 H, NH), 7.48 (s, 1 H, 17-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.6 (q, C-27), 16.5 (q, C-28), 17.6 (q, C-3), 20.0 (q, C-21), 20.4 (q, C-21′), 23.5 (t, C-34), 27.4 (d, C-20), 27.8 (q, C-22), 36.4 (d, C-2), 41.4 (t, C-6), 45.1 (q, C-35), 49.4 (d, C-59), 51.7 (q, C-36), 53.3 (d, C-24), 55.5 (t, C-34), 62.4 (d, C-19), 66.9 (t, C-18), 70.2 (d, C-30), 113.8 (d, C-17), 117.3 (d, C-15), 119.8 (d, C-13), 126.4 (d, C-10), 135.6 (s, C-12), 138.0 (s, C-7), 158.5 (s, C-16), 166.2 (s, C-11), 173.0, 174.2 (2s, C-7, C-13), 176.8 (s, C-1) ppm. HRMS (CI): calcd. for $C_{39}H_{59}N_4O_6$ $[M + H]^+$ 679.4434; found 679.4454.

Phenylpretubulysin Trifluoroacetate (1·Tfa): A mixture of *N*-methylated tetrapeptide **17** (66 mg, 97 μmol) and 1 M NaOH (0.20 mL, 0.20 mmol) in dioxane (1 mL) was stirred at 80 °C until complete saponification occurred (4 h). The solvent was removed in vacuo and the residue was dissolved in water, acidified to pH 1 with trifluoroacetic acid, and extracted thrice with EtOAc. The combined organic layers were dried with Na_2SO_4 and the solvent was evaporated in vacuo. Purification by flash chromatography (dichloromethane/MeOH, 9:1) provided the TFA salt of **1** (68 mg, 88 μmol, 91%) as a white solid and a mixture of rotamers; m.p. 110–112 °C. R_f = 0.30 (dichloromethane/MeOH, 9:1). $[\alpha]_D^{20}$ = –21.3 (*c* = 1.1, MeOH). **Major rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.80 (d, $^3J_{22,21}$ = 6.4 Hz, 3 H, 22-H), 0.89–1.00 (m, 6 H, 28-H, 29-H), 1.05 (d, $^3J_{22,21}$ = 6.7 Hz, 3 H, 22-H), 1.18 (d, $^3J_{3,2}$ = 7.0 Hz, 3 H, 3-H), 1.26 (m, 1 H, 27-H_a), 1.51–1.73 (m, 3 H, 4-H_a, 27-H_b, 33-H_a), 1.73–1.88 (m, 4 H, 4-H_b, 19-H_a, 32-H_a, 34-H_a), 1.88–2.03 (m, 5 H, 19-H_b, 21-H, 26-H, 33-H_b, 34-H_b), 2.15 (m, 1 H, 32-H_b), 2.48 (m, 1 H, 18-H_a), 2.51–2.62 (m, 2 H, 2-H, 18-H_b), 2.73 (s, 3 H, 36-H), 2.89 (d, $^3J_{6,5}$ = 6.9 Hz, 2 H, 6-H), 3.06 (m, 1 H, 35-H_a), 3.13 (s, 3 H, 23-H), 3.49 (m, 1 H, 35-H_b), 3.78 (dd, $^3J_{31,32a}$ = 11.8, $^3J_{31,32b}$ = 2.4 Hz, 1 H, 31-H), 4.18 (br. s, 1 H, 20-H), 4.39 (m, 1 H, 5-H), 4.71 (d, $^3J_{25,26}$ = 7.9 Hz, 1 H, 25-H), 7.16 (m, 1 H, 10-H), 7.20–7.28 (m, 4 H, 8-H, 9-H), 7.31–7.36 (m, 2 H, 14-H, 15-H), 7.50–7.58 (m, 2 H, 13-H, 17-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.3 (q, C-28), 16.2 (q, C-29), 18.6 (q, C-3), 20.3 (q, C-22), 20.6

(q, C-22′), 22.3 (t, C-33), 24.0 (t, C-34), 25.5 (t, C-27), 30.2 (t, C-32), 30.5 (q, C-23), 31.3 (d, C-21), 32.6 (t, C-19), 34.1 (t, C-18), 37.5 (d, C-26), 38.0 (d, C-2), 39.2 (t, C-4), 42.4 (q, C-36), 42.9 (t, C-6), 51.1 (d, C-5), 56.2 (t, C-35), 56.3 (d, C-25), 61.7 (d, C-20), 68.1 (d, C-31), 118.3 (q, $^2J_{C,F}$ = 1170 Hz, CF_3COOH), 125.9 (d, C-13), 127.4 (d, C-10), 128.3 (d, C-17), 129.3 (d, C-8), 129.6 (d, C-14), 130.5 (d, C-9), 132.5 (d, C-15), 136.2 (s, C-12), 139.9 (s, C-7), 143.6 (s, C-16), 163.0 (q, $^3J_{C,F}$ = 132 Hz, CF_3COOH), 169.3 (s, C-11), 170.3 (s, C-30), 174.5 (s, C-24), 180.2 (s, C-1) ppm. **Minor rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.09 (d, $^3J_{22,21}$ = 6.7 Hz, 3 H, 22-H), 1.36 (d, $^3J_{3,2}$ = 6.4 Hz, 3 H, 3-H), 2.21 (m, 1 H, 32-H_b), 2.73 (s, 3 H, 36-H), 3.13 (s, 3 H, 23-H), 3.49 (m, 1 H, 35-H_b), 3.85 (dd, $^2J_{31,32a}$ = 11.8, $^2J_{31,32b}$ = 2.4 Hz, 1 H, 31-H), 5.05 (d, $^3J_{25,26}$ = 5.5 Hz, 1 H, 25-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.7 (q, C-28), 16.5 (q, C-29), 21.1 (q, C-22), 28.4 (q, C-23), 43.0 (t, C-6), 51.2 (d, C-2), 55.4 (d, C-25), 65.1 (d, C-20), 125.7 (d, C-13), 128.6 (d, C-17), 132.3 (d, C-15), 143.4 (s, C-16), 173.7 (s, C-24) ppm. HRMS (CI): calcd. for $C_{39}H_{59}N_4O_5$ $[M + H]^+$ 663.4485; found 663.4474.

Phenoxypretubulysin Trifluoroacetate (2·Tfa): According to the preparation of **1·Tfa**, compound **2·Tfa** was obtained by saponification of **18** (103 mg, 0.15 mmol) with 1 M NaOH (0.30 mL, 0.30 mmol) in dioxane (1.5 mL) to yield **2·Tfa** (112 mg, 0.14 mmol, 94%) as a white solid and a mixture of rotamers; m.p. 116–118 °C. R_f = 0.19 (dichloromethane/MeOH, 9:1). $[\alpha]_D^{20}$ = –32.5 (*c* = 1.0, MeOH). **Major rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (d, $^3J_{21,20}$ = 6.5 Hz, 3 H, 21-H), 0.92 (t, $^3J_{27,26}$ = 7.6 Hz, 3 H, 27-H), 1.02 (d, $^3J_{28,25}$ = 6.5 Hz, 3 H, 28-H), 1.07 (d, $^3J_{21,20}$ = 6.3 Hz, 3 H, 21-H), 1.18 (d, $^3J_{3,2}$ = 7.0 Hz, 3 H, 3-H), 1.22 (m, 1 H, 26-H_a), 1.56–1.71 (m, 3 H, 4-H_a, 26-H_a, 32-H_a), 1.76–1.82 (m, 2 H, 31-H_a, 33-H_a), 1.84–1.94 (m, 3 H, 25-H, 32-H_b, 33-H_b), 1.96–2.08 (m, 2 H, 4-H_b, 20-H), 2.13 (m, 1 H, 31-H), 2.57 (m, 1 H, 2-H), 2.72 (s, 3 H, 35-H), 2.88 (d, $^3J_{6,5}$ = 6.8 Hz, 2 H, 6-H), 3.07 (ddd, $^2J_{34a34b}$ = $^3J_{34a,33a}$ = 12.8, $^3J_{34a,33b}$ = 2.6 Hz, 1 H, 34-H_a), 3.15 (s, 3 H, 22-H), 3.48 (m, 1 H, 34-H_b), 3.75 (dd, $^3J_{30,31a}$ = 12.0, $^3J_{30,31b}$ = 2.8 Hz, 1 H, 30-H), 4.17 (m, 1 H, 5-H), 4.61–4.63 (m, 2 H, 19-H, 24-H), 7.03 (d, $^3J_{15,14}$ = 7.2 Hz, 1 H, 15-H), 7.16 (m, 1 H, 10-H), 7.20–7.35 (m, 7 H, 9-H, 13-H, 14-H, 17-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2 (q, C-27), 15.6 (q, C-28), 18.5 (q, C-3), 20.1 (q, C-21), 20.4 (q, C-21′), 22.3 (t, C-32), 24.1 (t, C-33), 26.0 (t, C-26), 28.1 (d, C-20), 30.1 (t, C-31), 31.2 (q, C-22, C-H, COSY), 37.7 (d, C-25), 37.9 (d, C-2), 39.2 (t, C-4), 42.3 (t, C-6), 42.8 (q, C-35), 51.1 (d, C-5), 56.0 (d, C-24), 56.2 (t, C-34), 60.5 (d, C-19), 67.6 (t, C-18), 68.1 (d, C-30), 114.0 (d, C-17), 119.0 (d, C-15), 120.8 (d, C-13), 127.4 (d, C-10), 129.3, 130.5 (2d, C-8, C-9), 130.6 (d, C-14), 137.7 (s, C-12), 139.9 (s, C-7), 160.0 (s, C-16), 169.2, 169.8 (2s, C-11, C-29), 175.0 (s, C-23), 180.0 (s, C-1) ppm. **Minor rotamer:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.14 (d, $^3J_{21,20}$ = 6.9 Hz, 3 H, 21-H), 2.54 (s, 3 H, 35-H), 2.78 (s, 3 H, 22-H), 2.97 ($^2J_{34a34b}$ = $^3J_{34a,33a}$ = 12.7, $^3J_{34a,33b}$ = 2.9 Hz, 1 H, 34-H_a), 3.42 (m, 1 H, 34-H_b), 3.81 (dd, $^3J_{30,31a}$ = 11.9, $^3J_{30,31b}$ = 3.1 Hz, 1 H, 30-H), 4.06–4.08 (m, 2 H, 18-H_a, 19-H), 4.26 (m, 1 H, 18-H_b), 7.10 (d, $^3J_{15,14}$ = 7.2 Hz, 1 H, 15-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.5 (q, C-27), 16.4 (q, C-28), 20.3 (q, C-21), 20.8 (q, C-21′), 39.3 (t, C-4), 42.4 (t, C-6), 43.0 (q, C-35), 55.3 (d, C-5), 64.0 (d, C-19), 120.7 (d, C-13), 137.5 (s, C-12), 139.9 (s, C-7), 168.8, 169.5 (2s, C-11, C-29), 174.5 (s, C-23) ppm. HRMS (CI): calcd. for $C_{38}H_{57}N_4O_6$ $[M + H]^+$ 665.4278; found 665.4251.

Acknowledgments

Financial support by the Deutsche Forschungsgemeinschaft (DFG) (FOR 1406, Ka 880/10-1) is gratefully acknowledged. We also thank Jennifer Herrmann at the Department of Pharmaceutical Biotechnology for investigation of the biological activities.

[1] a) M. R. Tomblyn, M. S. Tallman, *Semin. Oncol.* **2003**, *30*, 502–508; b) L. M. Smith, A. Nesterova, S. C. Alley, M. Y. Torgov, P. J. Carter, *Mol. Cancer Ther.* **2006**, *5*, 1474–1482; c) S. O. Doronina, T. D. Bovee, D. W. Meyer, J. B. Miyamoto, M. E. Anderson, C. A. Morris-Tilden, P. D. Senter, *Bioconjugate Chem.* **2008**, *19*, 1960–1963.

[2] C. Liu, B. M. Tadayoni, L. A. Bourret, K. M. Mattocks, S. M. Derr, W. C. Widdison, N. L. Kederska, P. D. Ariniello, V. S. Goldmacher, J. M. Lambert, W. A. Blättler, R. V. Chari, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 8618–8623.

[3] Reviews: a) G. Höfle, N. Glaser, T. Leibold, U. Karama, F. Sasse, H. Steinmetz, *Pure Appl. Chem.* **2003**, *75*, 167–178; b) A. Dömling, W. Richter, *Mol. Diversity* **2005**, *9*, 141–147.

[4] a) S. O. Doronina, B. E. Toki, M. Y. Torgov, B. A. Mendelsohn, C. G. Cerveny, D. F. Chace, R. L. DeBlanc, R. P. Gearing, T. D. Bovee, C. B. Siegall, J. A. Francisco, A. F. Wahl, D. L. Meyer, S. D. Senter, *Nat. Biotechnol.* **2003**, *21*, 778–784; b) S. D. Shnyder, P. A. Cooper, N. J. Millington, G. R. Pettit, M. C. Bibby, *Int. J. Oncol.* **2007**, *31*, 353–360.

[5] a) G. R. Pettit, Y. Kamano, C. L. Herald, A. A. Tuinman, F. E. Boettner, H. Kizu, J. M. Schmidt, L. Bavczynskyj, K. B. Tomer, R. J. Bontems, *J. Biol. Chem.* **1990**, *265*, 17141–17149; b) G. R. Pettit, Y. Kamano, C. L. Herald, Y. Fujii, H. Kizu, M. R. Boyd, F. E. Boettner, D. L. Doubek, J. M. Schmidt, J. C. Chapuis, *Tetrahedron* **1993**, *49*, 9151–9170.

[6] G. Kaur, M. Hollingshead, S. Holbeck, V. Schauer-Vukašinović, R. F. Camalier, A. Dömling, S. Agarwal, *Biochem. J.* **2006**, *396*, 235–242.

[7] a) F. Sasse, H. Steinmetz, J. Heil, G. Höfle, H. Reichenbach, *J. Antibiot.* **2000**, *53*, 879–885; b) H. Steinmetz, N. Glaser, E. Herdtweck, F. Sasse, H. Reichenbach, G. Höfle, *Angew. Chem.* **2004**, *116*, 4996–5000; *Angew. Chem. Int. Ed.* **2004**, *43*, 4888–4892.

[8] Y. Chai, D. Pistorius, A. Ullrich, K. J. Weissman, U. Kazmaier, R. Müller, *Chem. Biol.* **2010**, *17*, 296–309.

[9] a) S. C. Wenzel, R. Müller, *Curr. Opin. Biotechnol.* **2005**, *16*, 594–606; b) H. B. Bode, R. Müller, *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 577–588; c) S. C. Wenzel, R. Müller, *Nat. Prod. Rep.* **2007**, *24*, 1211–1224.

[10] a) A. Sandmann, F. Sasse, R. Müller, *Chem. Biol.* **2004**, *11*, 1071–1079; b) A. Ullrich, Y. Chai, D. Pistorius, Y. A. Elnakady, J. E. Herrmann, K. J. Weissman, U. Kazmaier, R. Müller, *Angew. Chem.* **2009**, *121*, 4486–4489; *Angew. Chem. Int. Ed.* **2009**, *48*, 4422–4425.

[11] M. W. Khalil, F. Sasse, H. Lünsdorf, Y. A. Elnakady, H. Reichenbach, *ChemBioChem* **2006**, *7*, 678–683.

[12] K. Kubicek, S. K. Grimm, J. Orts, F. Sasse, T. Carlomagno, *Angew. Chem.* **2010**, *122*, 4919–4922; *Angew. Chem. Int. Ed.* **2010**, *49*, 4809–4812.

[13] H. M. Peltier, J. P. McMahon, A. W. Patterson, J. A. Ellman, *J. Am. Chem. Soc.* **2006**, *128*, 16018–16019.

[14] O. Pando, S. Dörner, R. Preusentanz, A. Denkert, A. Porzel, W. Richter, L. Wessjohann, *Org. Lett.* **2009**, *11*, 5567–5569.

[15] G. Höfle, T. Leibold, H. Steinmetz, DE 10008089, **2001** [*Chem. Abstr.* **2001**, *135*, 331296].

[16] a) A. Dömling, B. Henkel, B. Beck, WO 2004005269, **2004**, [*Chem. Abstr.* **2004**, *140*, 94054]; b) A. Dömling, B. Henkel, B. Beck, K. Illgen, S. Sakamuri, S. Menon, WO 2004005327, **2004**, [*Chem. Abstr.* **2004**, *140*, 94300].

[17] T. Shibue, T. Hirai, I. Okamoto, N. Morita, H. Masu, I. Azumaya, O. Tamura, *Chem. Eur. J.* **2010**, *16*, 11678–11688.

[18] a) D. Neri, G. Fossati, M. Zanda, *ChemMedChem* **2006**, *1*, 175–180; b) T. Shibue, I. Okamoto, N. Morita, H. Morita, Y. Hirasawa, T. Hosoya, O. Tamura, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 431–434.

[19] a) P. Wipf, T. Takada, M. J. Rishel, *Org. Lett.* **2004**, *6*, 4057–4060; b) S. P. Shankar, M. Sani, G. Terraneo, M. Zanda, *Syn-*

lett **2009**, 1341–1345; c) S. Chandrasekhar, B. Mahipal, M. Kavitha, *J. Org. Chem.* **2009**, *74*, 9531–9534.
[20] a) Z. Wang, P. A. McPherson, B. S. Raccor, R. Balachandran, G. Zhu, B. W. Day, A. Vogt, P. Wipf, *Chem. Biol. Drug Des.* **2007**, *70*, 75–86; b) P. Wipf, Z. Wang, *Org. Lett.* **2007**, *9*, 1605–1607.
[21] a) A. W. Patterson, H. M. Peltier, F. Sasse, J. A. Ellman, *Chem. Eur. J.* **2007**, *13*, 9534–9541; b) A. W. Patterson, H. M. Peltier, J. A. Ellman, *J. Org. Chem.* **2008**, *73*, 4362–4369.
[22] a) A. Dömling, W. Richter, *Mol. Diversity* **2005**, *9*, 141–147; b) A. Dömling, B. Beck, U. Eichelberger, S. Sakamuri, S. Menon, Q.-Z. Chen, Y. Lu, L. A. Wessjohann, *Angew. Chem.* **2006**, *118*, 7393–7397; *Angew. Chem. Int. Ed.* **2006**, *45*, 7235–7239; c) M. Sani, G. Fossati, F. Huguenot, M. Zanda, *Angew. Chem.* **2007**, *119*, 3596–3599; *Angew. Chem. Int. Ed.* **2007**, *46*, 3526–3529; d) B. Raghavan, R. Balasubramanian, J. C. Steele, D. L. Sackett, R. A. Fecik, *J. Med. Chem.* **2008**, *51*, 1530–1533.
[23] A. Ullrich, J. Herrmann, R. Müller, U. Kazmaier, *Eur. J. Org. Chem.* **2009**, 6367–6378.
[24] V. Dvornikovs, D. B. Smithrud, *J. Org. Chem.* **2002**, *67*, 2160–2167.
[25] F. Picard, S. Barassin, A. Mokhtarian, R. W. Hartmann, *J. Med. Chem.* **2002**, *45*, 3406–3417.
[26] A. Sutherland, C. L. Willis, *J. Org. Chem.* **1998**, *63*, 7764–7769.
[27] S. D. Lepore, Y. He, *J. Org. Chem.* **2003**, *68*, 8261–8263.
[28] H. Lipshutz, D. W. Chung, B. Rich, R. Corral, *Org. Lett.* **2006**, *8*, 5069–5072.
[29] P. Li, J. C. Xu, *J. Org. Chem.* **2000**, *65*, 2951–2958.

Received: February 2, 2011
Published Online: April 18, 2011

