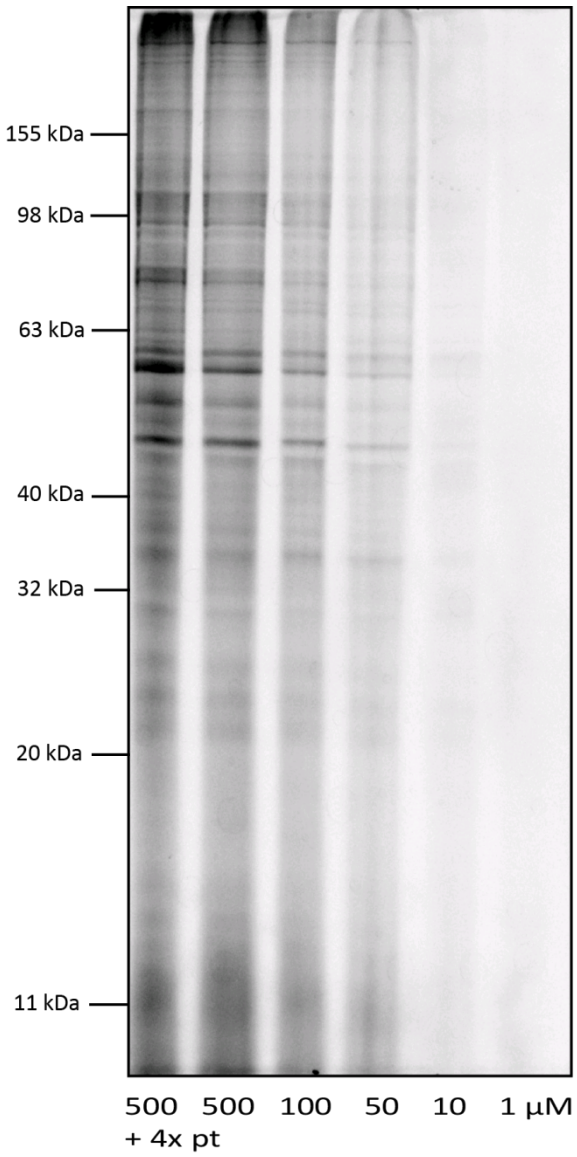
Electronic Supplementary Material (ESI) for Molecular BioSystems

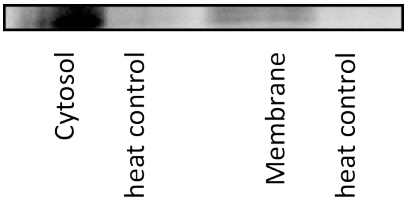
Supporting Information

Pretubulysin derived probes as novel tools for monitoring the microtubule network via Activity-Based Protein Profiling and Fluorescence Microscopy

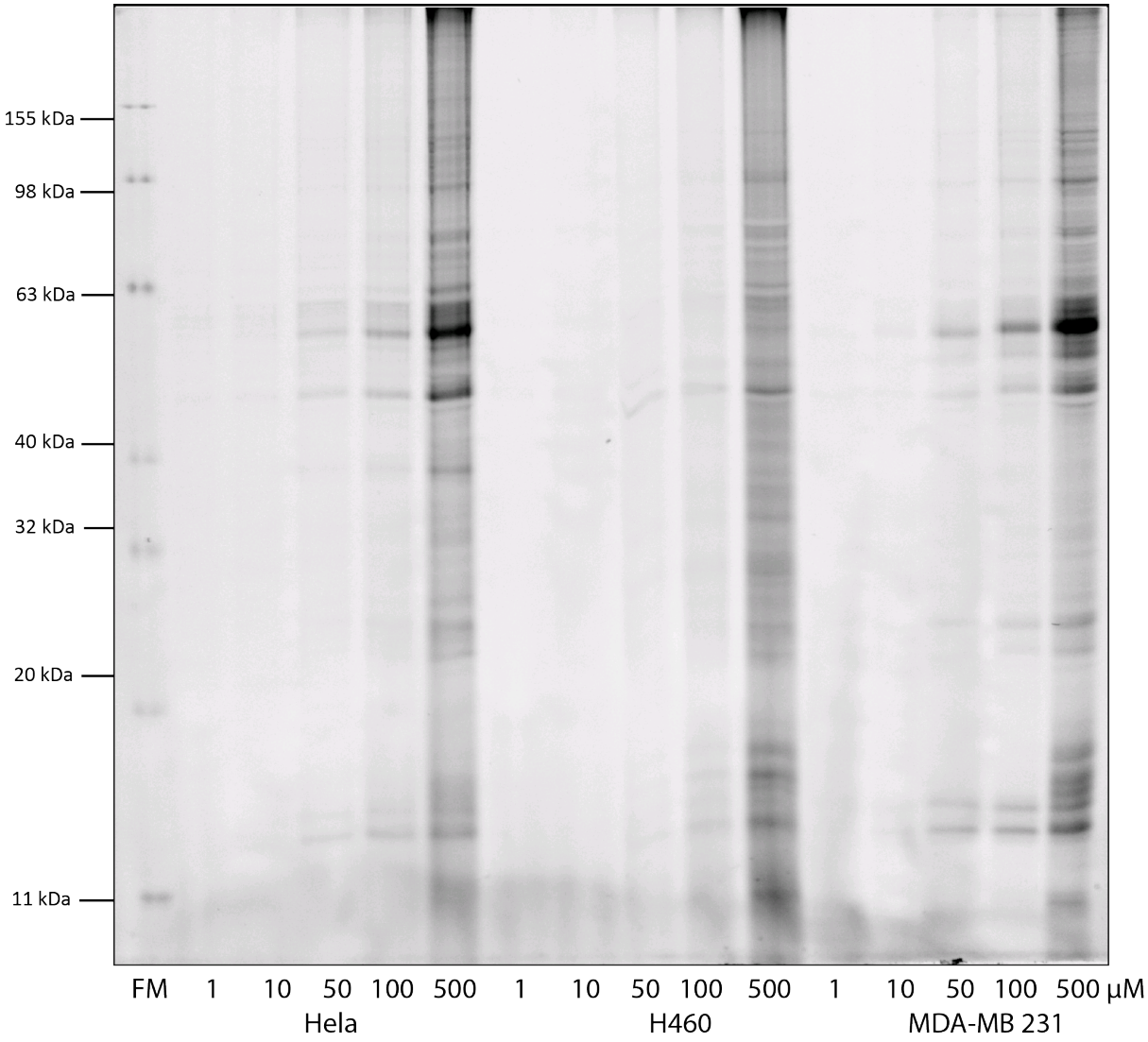
Jürgen Eirich, Jens L. Burkhart, Angelika Ullrich, Georg C. Rudolf, Angelika Vollmar, Stefan Zahler, Uli Kazmaier and Stephan A. Sieber



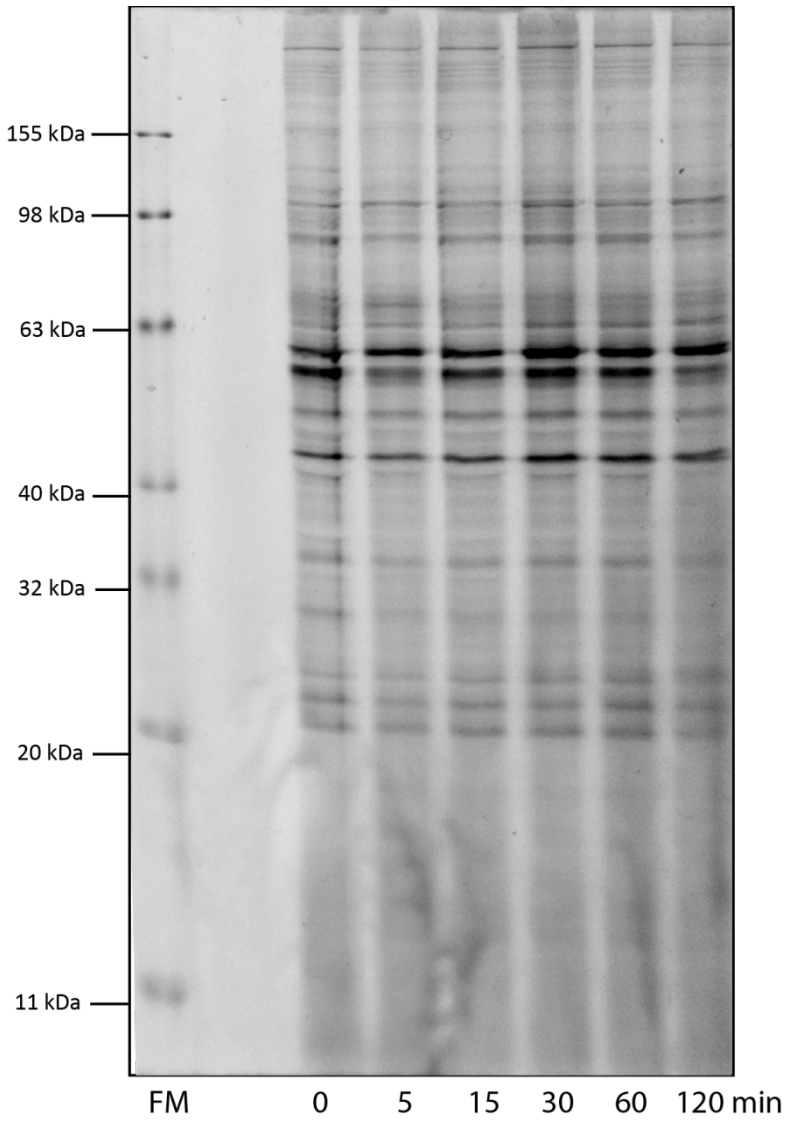
Supporting Figure 1: Concentration dependent *in situ* labeling (1 h incubation, 1 h UV irradiation) of membrane proteins in living Hela cells with compound **4**.



Supporting Figure 2: Heat controls of *in vitro* labeling of cytosolic and membrane proteins at about 60 kDa in Hela cell lysate with compound **4** (10 µM).



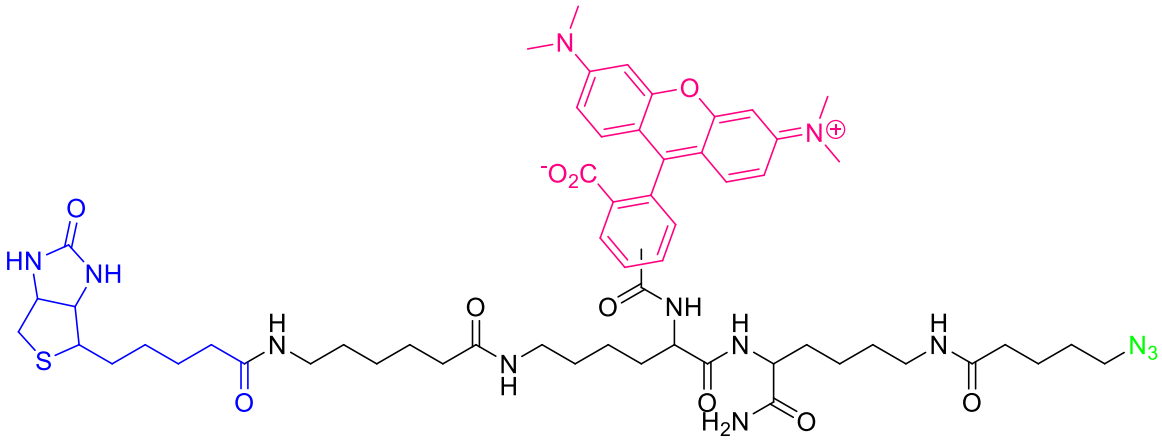
Supporting Figure 3: Concentration dependent *in situ* labeling of cytosolic proteins in different cell lines with probe **5** (10 µM).



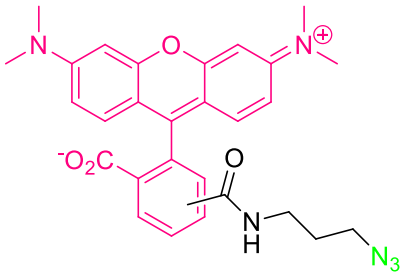
Supporting Figure 4: Time dependent *in situ* labeling: varying preincubation time of living Hela cells with compound **4** (10 µM) before UV irradiation (1 h).

	Score	Coverage	# Peptides	# PSM	Peak Area
H460 +	73.99	50.34	14	20	4.338E8
H460 -	8.10	6.29	2	3	3.134E7
Hela +	39.19	25.17	9	12	6.602E7
Hela -		0.00			0.000E0
Jurkat +	92.40	45.17	13	26	5.553E8
Jurkat -		0.00			5.069E7
MDA-MB +	53.82	44.27	13	14	1.823E8
MDA-MB -		0.00			0.000E0

Supporting Table 1: Mass spec data for tubulin, beta, 2 [Homo sapiens] (5174735;IPI00007752.1) selectively enriched and identified in different cell lines, Peak Areas calculated via Precursor Ions Area Detector of Thermo Proteome Discoverer 1.3, + Sample labeled with UV probe prior to biotin enrichment, - Sample NOT labeled with UV probe prior to biotin enrichment.



biotin-rhodamine-azide **15**



rhodamine-azide

Supporting Scheme 1: Molecular structure of Rhodamine Azide (RhN_3) used for in-gel fluorescent scanning and fluorescent microscopy and the Trifunctional Linker **15** (biotin-rhodamine- (alkyl)azide) used for biotin avidin enrichment.

Trifunctional linker **15** was synthesized according to standard solid phase peptide synthesis (SPPS) protocols[1] *via* Fmoc/tBu strategy[2] starting on Fmoc-Rink Amid MBHA resin[3]. The protected building blocks were deprotected via TFA (2 % in DCM) and were coupled by means of TBTU[4]/HOBt[5] and DIPEA[6] in following order: a) Fmoc-Lys(MTT)-OH[7] b) 5-Azido-pentanoic acid[8] c) Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH[7] d) 5-(and-6)-carboxytetramethylrhodamine succinimidyl ester[9]. The peptidic compound was cleaved from the solid support with TFA (95 % in H_2O, 2 h at ambient temperature) and was precipitated in cold diethyl ether at -20 °C. The solid residue was purified *via* RP-HPLC (XBridge BEH C18 5 µm 30 x 150 mm, linear gradient 2 % - 98 % acetonitrile / H_2O with 0.1 % TFA in 30 min, UV-Vis detection at 550 nm, RT 10.2 – 10.8 min) and yielded after lyophilisation 59.6 mg purple powder (51.8 µmol, 24 % overall yield).

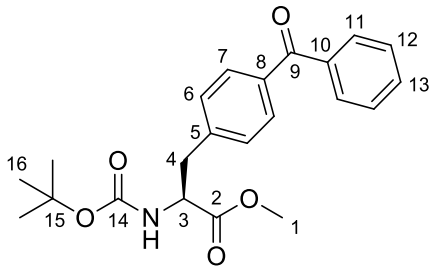
HRMS (ESI)	calculated	found
$[M+H]^+ = C_{85}H_{80}N_{13}O_{10}S^+$	1150.5866	1150.5850
$[M+2H]^{2+} = C_{85}H_{81}N_{13}O_{10}S^{2+}$	575.7970	575.7965

Synthetic procedures

(*S*)-methyl 3-(4-benzoylphenyl)-2-(*tert*-butoxycarbonylamino)propanoate (7)

A solution of Boc-protected iodophenylalanine methylester **6** (785 mg, 2.07 mmol), K_2CO_3 (857 mg, 6.21 mmol), *bis*(triphenylphosphine)palladium(II) dichloride (73 mg, 0.10 mmol) and phenylboronic acid (278 mg, 2.28 mmol) in anisole (20 mL) was stirred under CO-atmosphere at 80°C for 19 h. After cooling to room temperature the reaction mixture was diluted with diethyl ether (80 mL), washed with water and brine, dried with Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (hexanes/ethyl acetate, 9:1, 7:3) to yield **7** (686 mg, 1.79 mmol, 83%) as a yellowish oil.

[tlc: hexanes/ethyl acetate = 8:2, R_f(**7**) = 0.14]



7

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 1.42 (s, 9 H, 16-H), 3.11 (dd, $^2J_{4a,4b}$ = 13.5 Hz, $^3J_{4a,3}$ = 6.1 Hz, 1 H, 4-H_a), 3.23 (dd, $^2J_{4b,4a}$ = 13.6 Hz, $^3J_{4b,3}$ = 5.6 Hz, 1 H, 4-H_b), 3.74 (s, 3 H, 1-H), 4.64 (m, 1 H, 3-H), 5.03 ($^3J_{NH,3}$ = 7.5 Hz, 1 H, NH), 7.25 (d, $^3J_{6,7}$ = 8.1 Hz, 2 H, 6-H), 7.48 (dd, $^3J_{12,11}$ = $^3J_{12,13}$ = 7.6 Hz, 2 H, 12-H), 7.59 (tt, $^3J_{13,12}$ = 7.4 Hz, $^4J_{13,11}$ = 1.3 Hz, 1 H, 13-H), 7.75 (d, $^3J_{7,6}$ = 8.2 Hz, 2 H, 7-H), 7.77 (dd, $^3J_{11,12}$ = 8.4 Hz, $^4J_{11,13}$ = 1.4 Hz, 2 H, 11-H).

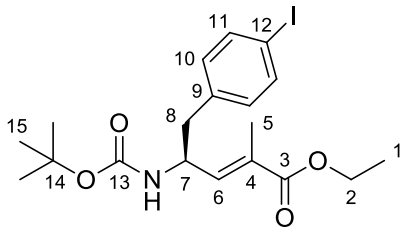
^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 28.3 (q, C-16), 38.4 (t, C-4), 52.4 (q, C-1), 54.2 (d, C-3), 80.1 (s, C-15), 128.3 (d, C-12), 129.3 (d, C-6), 129.9 (d, C-11), 130.3 (d, C-7), 132.4 (d, C-13), 136.3 (s, C-8), 137.6 (s, C-10), 141.1 (s, C-5), 155.0 (s, C-14), 172.0 (s, C-2), 196.3 (s, C-9).

optical rotation: $[\alpha]^{20}_D$ = +50.3° (c = 2.0, $CHCl_3$) [Lit.: $[\alpha]^{20}_D$ = +51.0° (c = 2.0, $CHCl_3$)[10]

(*S*,*E*)-ethyl 4-(*tert*-butoxycarbonylamino)-5-(4-iodophenyl)-2-methylpent-2-enoate (9)

A solution of DIBALH (1 M) in hexane (4.20 mL, 4.20 mmol) was added dropwise at –78°C to a solution of Boc-protected iodophenylalanine methylester **6** (850 mg, 2.10 mmol) in dry dichloromethane (12 mL). After stirring the reaction mixture for 1 h at –78°C, phosphonium salt **8** (1.02 g, 2.31 mmol) and KO*t*Bu (259 mg, 2.31 mmol) were added and the reaction mixture 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 (hexanes/ethyl acetate, 8:2) to yield **9** (805 mg, 1.75 mmol, 83%) as a white solid, m.p. 112–113°C.

[tlc: hexanes/ethyl acetate = 8:2, R_f(**9**) = 0.28]



9

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 1.28 (t, $^3J_{1,2}$ = 7.1 Hz, 1-H), 1.40 (s, 9 H, 15-H), 1.71 (d, $^4J_{5,6}$ = 1.3 Hz, 3 H, 5-H), 2.72 (dd, $^2J_{8a,8b}$ = 13.5 Hz, $^3J_{8a,7}$ = 7.0 Hz, 1 H, 8-H_a), 2.86 (dd, $^2J_{8b,8a}$ = 13.3 Hz, $^3J_{8b,7}$ = 5.5 Hz, 1 H, 8-H_b), 4.18 (q, $^3J_{2,1}$ = 7.1 Hz, 2 H, 2-H), 4.50–4.66 (m, 2 H, 7-H, NH), 6.47 (dq, $^3J_{6,7}$ = 9.0 Hz, $^4J_{6,5}$ = 1.4 Hz, 1 H, 6-H), 6.92 (d, $^3J_{10,11}$ = 8.2 Hz, 2 H, 10-H), 7.60 (d, $^3J_{11,10}$ = 8.2 Hz, 1 H, 11-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 12.7 (q, C-5), 14.2 (q, C-1), 28.3 (q, C-15), 40.7 (t, C-8), 49.9 (d, C-7), 60.8 (t, C-2), 79.8 (s, C-14), 92.0 (d, C-12), 129.8 (s, C-4), 131.5 (d, C-19), 136.4 (s, C-9), 137.5 (d, C-11), 139.5 (s, C-6), 154.9 (s, C-13), 167.6 (s, C-3).

optical rotation: $[\alpha]^{21}_D$ = +21.8° (c = 0.9, >95% *E,* $CHCl_3$)

HRMS (CI)	calculated	found
$C_{19}H_{27}NO_4I$ $[M+H]^+$	460.0985	460.0993

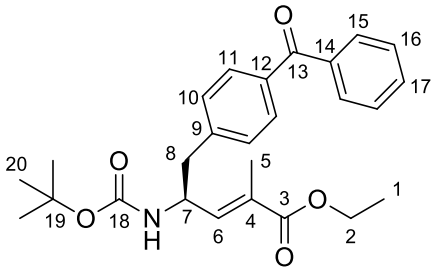
elemental analysis:

$C_{19}H_{26}NO_4I$	calculated	C 49.68	H 5.71	N 3.05
(459.32)	found	C 50.12	H 5.64	N 2.74

(*S*,*E*)-ethyl 5-(4-benzoylphenyl)-4-(*tert*-butoxycarbonylamino)-2-methylpent-2-enoate (10)

A solution of **9** (88 mg, 0.19 mmol), K_2CO_3 (79 mg, 0.57 mmol), *bis*(triphenylphosphine)palladium(II) dichloride (7 mg, 10 µmol) and phenylboronic acid (27 mg, 0.22 mmol) in anisole (2 mL) was stirred under CO-atmosphere at 80°C for 17 h. After cooling to room temperature the reaction mixture was diluted with diethyl ether (80 mL), washed with water and brine, dried with Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (hexanes/ethyl acetate, 8:2) to yield **10** (81 mg, 0.19 mmol, 97%) as a reddish solid, m.p. 76–78°C.

[tlc: hexanes/ethyl acetate = 8:2, R_f(**10**) = 0.13]



10

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 1.28 (t, $^3J_{1,2}$ = 7.1 Hz, 3 H, 1-H), 1.40 (s, 9 H, 15-H), 1.73 (d, $^4J_{5,6}$ = 1.4 Hz, 3 H, 5-H), 2.87 (dd, $^2J_{8a,8b}$ = 13.3 Hz, $^3J_{8a,7}$ = 7.0 Hz, 1 H, 8-H_a), 3.02 (dd, $^2J_{8b,8a}$ = 13.0 Hz, $^3J_{8b,7}$ = 5.7 Hz, 1 H, 8-H_b), 4.18 (q, $^3J_{2,1}$ = 7.1 Hz, 2 H, 2-H), 4.60–4.76 (m, 2 H, 7-H, NH), 6.53 (dq, $^3J_{6,7}$ = 9.0 Hz, $^4J_{6,5}$ = 1.4 Hz, 1 H, 6-H), 7.29 (d, $^3J_{10,11}$ = 8.2 Hz, 2 H, 10-H), 7.47 (dd, $^3J_{16,15}$ = $^3J_{16,17}$ = 7.6 Hz, 2 H, 16-H), 7.58 (tt, $^3J_{17,16}$ = 7.4 Hz, $^4J_{17,15}$ = 1.3 Hz, 1 H, 17-H), 7.74 (d, $^3J_{11,10}$ = 8.2 Hz, 2 H, 11-H), 7.77 (dd, $^3J_{15,16}$ = 8.4 Hz, $^4J_{15,17}$ = 1.4 Hz, 2 H, 15-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 12.7 (q, C-5), 14.2 (q, C-1), 28.3 (q, C-20), 41.3 (t, C-8), 50.0 (d, C-7), 60.8 (t, C-2), 80.0 (s, C-19), 128.2 (d, C-16), 129.5 (d, C-10), 129.9 (s, d, C-4, C-15), 130.3 (d, C-11), 132.3 (d, C-17), 136.0 (s, C-12), 137.6 (s, C-14), 139.5 (s, C-6), 141.8 (s, C-9), 155.0 (s, C-18), 172.0 (s, C-3), 196.3 (s, C-13).

optical rotation: $[\alpha]^{21}_D$ = +25.4° (c = 1.1, $CHCl_3$)

HRMS (CI)	calculated	found
$C_{22}H_{24}NO_5$ $[M–C_4H_8+H]^+$	382.1654	382.1667

elemental analysis:

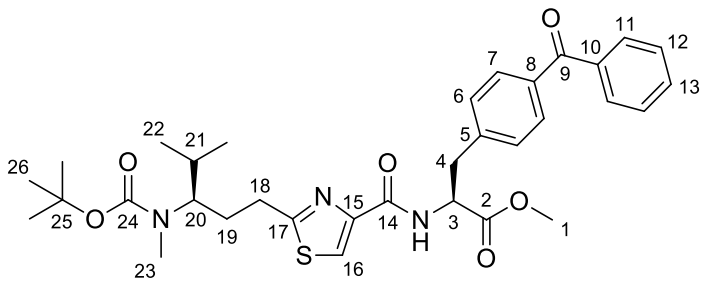
$C_{26}H_{31}NO_5$	calculated	C 71.37	H 7.14	N 3.20
(437.53)	found	C 70.75	H 7.15	N 2.85

(*S*)-methyl 3-(4-benzoylphenyl)-2-(2-((*R*)-3-(*tert*-butoxycarbonyl(methyl)amino)-4-methylpentyl)thiazole-4-carboxamido)propanoate (11a)

HCl in dioxane (1.28 mL, 5.10 mmol, 4 M) was added at 0°C to **7** (196 mg, 0.51 mmol). The solvent was evaporated in vacuum after complete deprotection (30 min, tlc monitoring) and the hydrochloride salt was dried in high vacuum.

Isobutyl chloroformate (68 µL, 0.51 mmol) was added dropwise at –20°C to a solution of Boc-protected tubuvaline **BocTuvOH**[11] (157 mg, 0.46 mmol) and NMM (127 µL, 1.15 mmol) in dry THF (5 mL). After 20 min, *N*-deprotected **7** was added and the mixture was allowed to warm to room temperature overnight. Water was added and the mixture was extracted with ethyl acetate (3x). The combined organic layers were washed with aqueous HCl (1 M), water, saturated aqueous $NaHCO_3$, water and brine, dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by flash chromatography (SiO_2, hexanes/ethyl acetate = 6:4) to yield dipeptide **11a** (238 mg, 0.39 mmol, 85% over 2 steps) as colourless oil and a mixture of rotamers.

[tlc: hexanes/ethyl acetate = 1:1, R_f(**11a**) = 0.25]



11a

major rotamer

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.81 (d, $^3J_{22,21}$ = 6.5 Hz, 3 H, 22-H), 0.83 (d, $^3J_{22',21}$ = 6.5 Hz, 3 H, 22'-H), 1.38 (s, 9 H, 26-H), 1.65 (m, 1 H, 21-H), 1.78 (m, 1 H, 19-H_a), 2.14 (m, 1 H, 19-H_b), 2.63 (s, 3 H, 23-H), 2.88 (m, 2 H, 18-H), 3.27 (dd, $^2J_{4a,4b}$ = 13.9 Hz, $^3J_{4a,3}$ = 6.4 Hz, 1 H, 4-H_a), 3.36 (dd, $^2J_{4b,4a}$ = 13.9 Hz, $^3J_{4b,3}$ = 6.4 Hz, 1 H, 4-H_b), 3.74 (s, 3 H, 1-H), 3.82 (m, 1 H, 20-H), 5.09 (m, 1 H, 3-H), 7.30 (d, $^3J_{6,7}$ = 8.1 Hz, 2 H, 6-H), 7.46 (dd, $^3J_{12,11}$ = $^3J_{12,13}$ = 7.6 Hz, 2 H, 12-H), 7.57 (t, $^3J_{13,12}$ = 7.4 Hz, 13-H), 7.73 (d, $^3J_{7,6}$ = 8.3 Hz, 2 H, 7-H), 7.75 (m, 2 H, 11-H), 7.79 ($^3J_{NH,3}$ = 8.0 Hz, 1 H, NH), 7.40 (s, 1 H, 16-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 19.6 (q, C-22), 19.9 (q, C-22'), 28.2 (q, C-23), 28.4 (q, C-26), 29.5 (t, C-19), 30.1 (t, C-18), 30.5 (d, C-21), 38.1 (t, C-4), 52.4 (q, C-1), 53.0 (d, C-3), 60.3 (d, C-20), 79.2 (s, C-25), 123.1 (d, C-16), 128.2 (d, C-12), 129.2 (d, C-6), 129.9 (d, C-11), 130.4 (d, C-7), 132.3 (d, C-13), 136.2 (s, C-8), 137.6 (s,

C-10), 141.1 (s, C-5), 148.7 (s, C-15), 156.4 (s, C-24), 160.6 (s, C-14), 170.8 (s, C-17), 171.4 (s, C-2), 196.2 (s, C-9).

minor rotamer (selected signals)

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.91 (d, $^{3}J_{22,21}$ = 6.5 Hz, 3 H, 22-H), 0.93 (d, $^{3}J_{22',21}$ = 6.5 Hz, 3 H, 22'-H), 1.44 (s, 9 H, 26-H), 2.66 (s, 3 H, 23-H), 3.28 (dd, $^{2}J_{4a,4b}$ = 13.7 Hz, $^{3}J_{4a,3}$ = 6.2 Hz, 1 H, 4-H_a), 3.64 (bs, 1 H, 20-H), 7.96 (s, 1 H, 16-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 20.1 (q, C-22), 20.3 (q, C-22'), 28.4 (q, C-23), 29.1 (t, C-19), 30.3 (t, C-18), 30.7 (d, C-21), 38.2 (t, C-4), 53.0 (d, C-3), 79.5 (s, C-25), 129.2 (d, C-6), 129.9 (d, C-11), 136.2 (s, C-8), 141.1 (s, C-5), 148.8 (s, C-15), 156.6 (s, C-24), 160.7 (s, C-14), 171.0 (s, C-17), 171.5 (s, C-2), 196.2 (s, C-9).

optical rotation: $[\alpha]^{21}_{D}$ = +9.4° (c = 0.6, $CHCl_3$)

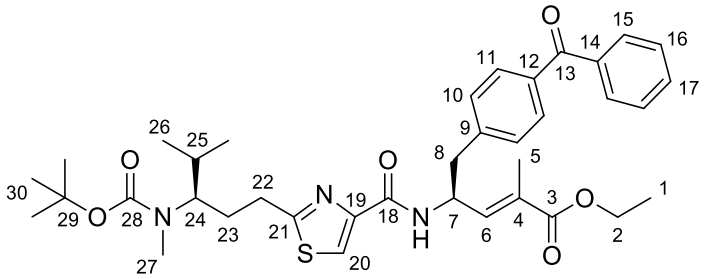
HRMS (CI)	calculated	found
$C_{33}H_{42}N_3O_6S$ $[M+H]^+$	608.2794	608.2751

(*S*,*E*)-ethyl 5-(4-benzoylphenyl)-4-(2-((*R*)-3-(*tert*-butoxycarbonyl(methyl)amino)-4-methylpentyl)thiazole-4-carboxamido)-2-methylpent-2-enoate (11b)

HCl in dioxane (1.63 mL, 6.50 mmol, 4 M) was added at 0°C to **10** (284 mg, 0.65 mmol). The solvent was evaporated in vacuum after complete deprotection (30 min, tlc monitoring) and the hydrochloride salt was dried in high vacuum.

Isobutyl chloroformate (86 µL, 0.65 mmol) was added dropwise at –20°C to a solution of Boc-protected tubuvaline **Boc-TuvOH**[11] (223 mg, 0.65 mmol) and NMM (180 µL, 1.63 mmol) in dry THF (6 mL). After 20 min, *N*-deprotected **10** was added and the mixture was allowed to warm to room temperature overnight. Water was added and the mixture was extracted with ethyl acetate (3x). The combined organic layers were washed with aqueous HCl (1 M), water, saturated aqueous $NaHCO_3$, water and brine, dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate = 7:3, 6:4) to yield dipeptide **11b** (394 mg, 0.60 mmol, 92% over 2 steps) as a white solid and a mixture of rotamers, m.p. 49–52°C.

[tlc: petroleum ether/ethyl acetate = 6:4, R_f(**11b**) = 0.19]



11b

major rotamer

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.84 (d, $^3J_{26,25}$ = 6.5 Hz, 6 H, 26-H), 1.27 (t, $^3J_{1,2}$ = 7.1 Hz, 3 H, 1-H), 1.40 (s, 9 H, 30-H), 1.67 (m, 1 H, 25-H), 1.77 (d, $^4J_{5,6}$ = 1.1 Hz, 3 H, 5-H), 1.81 (m 1 H, 23-H_a), 2.11 (m, 1 H, 23-H_b), 2.65 (s, 3 H, 27-H), 2.85 (m, 2 H, 22-H), 3.00 (dd, $^2J_{8a,8b}$ = 13.2 Hz, $^3J_{8a,7}$ = 7.8 Hz, 1 H, 8-H_a), 3.20 (dd, $^2J_{8b,8a}$ = 13.4 Hz, $^3J_{8b,7}$ = 6.2 Hz, 1 H, 8-H_b), 3.79 (m, 1 H, 24-H), 4.18 (q, $^3J_{2,1}$ = 7.1 Hz, 2 H, 2-H), 5.20 (m, 1 H, 7-H), 6.67 (dq, $^3J_{6,7}$ = 9.0 Hz, $^4J_{6,5}$ = 1.4 Hz, 1 H, 6-H), 7.35 (d, $^3J_{10,11}$ = 8.1 Hz, 2 H, 10-H), 7.46 (dd, $^3J_{16,15}$ = $^3J_{16,17}$ = 7.6 Hz, 2 H, 16-H), 7.52 (d $^3J_{NH,7}$ = 8.2 Hz, 1 H, NH), 7.57 (t, $^3J_{17,16}$ = 7.4 Hz, 1 H, 17-H), 7.73 (d, $^3J_{11,10}$ = 8.3 Hz, 2 H, 11-H), 7.75 (d, $^3J_{15,16}$ = 7.8 Hz, 2 H, 15-H), 7.92 (s, 1 H, 20-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 12.9 (q, C-5), 14.2 (q, C-1), 19.6 (q, C-26), 19.9 (q, C-26‘), 28.2 (q, C-27), 28.4 (q, C-30), 29.7 (t, C-23), 30.2 (t, C-22), 30.5 (d, C-25), 41.0 (t, C-8), 48.6 (d, C-7), 60.1 (d, C-24), 60.8 (t, C-2), 79.2 (s, C-29), 122.8 (d,

C-20), 128.2 (d, C-16), 129.4 (d, C-10), 129.9 (d, C-15), 130.3 (d, C-11), 130.8 (s, C-4), 132.3 (d, C-17), 136.0 (s, C-12), 137.7 (s, C-14), 138.5 (s, C-6), 141.8 (s, C-9), 149.0 (s, C-19), 156.5 (s, C-28), 160.2 (s, C-18), 167.5 (s, C-21), 170.9 (s, C-3), 196.3 (s, C-13).

minor rotamer (selected signals)

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.95 (d, $^{3}J_{26,25}$ = 6.5 Hz, 6 H, 26-H), = 0.97 (d, $^{3}J_{26',25}$ = 6.5 Hz, 6 H, 26'-H), 1.46 (s, 9 H, 30-H), 1.79 (d, $^{4}J_{5,6}$ = 1.1 Hz, 3 H, 5-H), 3.01 (dd, $^{2}J_{8a,8b}$ = 13.2 Hz, $^{3}J_{8a,7}$ = 7.8 Hz, 1 H, 8-H_a), 3.21 (dd, $^{2}J_{8b,8a}$ = 13.4 Hz, $^{3}J_{8b,7}$ = 6.2 Hz, 1 H, 8-H_b), 3.65 (bs, 1 H, 24-H), 4.19 (q, $^{3}J_{2,1}$ = 7.1 Hz, 2 H, 2-H), 6.68 (dq, $^{3}J_{6,7}$ = 9.0 Hz, $^{4}J_{6,5}$ = 1.4 Hz, 1 H, 6-H), 7.35 (d, $^{3}J_{10,11}$ = 8.1 Hz, 2 H, 10-H), 7.52 (d $^{3}J_{NH,7}$ = 8.4 Hz, 1 H, NH), 7.94 (s, 1 H, 20-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 20.1 (q, C-26), 20.3 (q, C-26'), 29.4 (t, C-23), 30.2 (t, C-22), 30.7 (d, C-25), 41.0 (t, C-8), 48.7 (d, C-7), 79.5 (s, C-29), 129.4 (d, C-10), 130.8 (s, C-4), 149.2 (s, C-19), 156.6 (s, C-28), 160.3 (s, C-18), 167.6 (s, C-21), 171.1 (s, C-3).

optical rotation: $[\alpha]^{21}_{D}$ = +8.6° (c = 1.1, $CHCl_3$)

HRMS (CI)	calculated	found
$C_{37}H_{47}N_3O_6S$ [M]	661.3186	661.3233

elemental analysis:

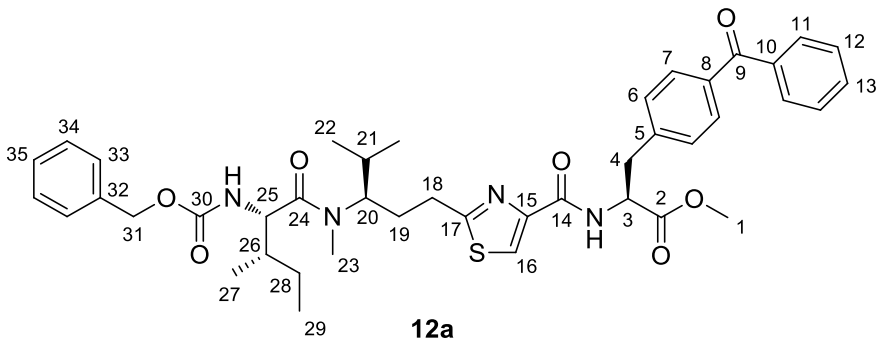
$C_{37}H_{47}N_3O_6S$	calculated	C 67.14	H 7.16	N 6.35
(661.85)	found	C 66.36	H 7.17	N 6.04

(*S*)-methyl 3-(4-benzoylphenyl)-2-(2-((*R*)-3-((2*S*,3*S*)-2-(benzyloxycarbonyl-amino)-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido) propanoate (12a)

HCl in dioxane (2.0 mL, 8.0 mmol, 4 M) was added at 0°C to dipeptide **11a** (182 mg, 0.30 mmol). The solvent was evaporated in vacuum after complete deprotection (30 min, tlc monitoring) and the hydrochloride salt was dried in high vacuum.

N-Deprotected **11a**, Cbz-protected L-isoleucine (90 mg, 0.33 mmol) and BEP (90 mg, 0.33 mmol) were dissolved in dry DCM (3 mL) and diisopropyl ethylamine (160 µL, 0.94 mmol) was added dropwise to this solution 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 DCM and washed with aqueous HCl (1 M), saturated aqueous $NaHCO_3$, water and brine, dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by flash chromatography (SiO_2, hexanes/ethyl acetate = 1:1) to yield tripeptide **12a** (195 mg, 0.26 mmol, 86% over 2 steps) as a white solid and a mixture of rotamers, m.p. 58–60°C.

[tlc: hexanes/ethyl acetate = 1:1, R_f(**12a**) = 0.18]



12a

major rotamer

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.75 (d, $^3J_{22,21}$ = 6.6 Hz, 3 H, 22-H), 0.85 (t, $^3J_{29,28}$ = 7.3 Hz, 3 H, 29-H), 0.94 (d, $^3J_{22',21}$ = 6.5 Hz, 3 H, 22'-H), 0.98 (d, $^3J_{27,26}$ = 6.7 Hz, 3 H, 27-H), 1.10 (m, 1 H, 28-H_a), 1.58 (m, 1 H, 28-H_b), 1.67–1.77 (m, 2 H, 21-H, 26-H), 1.84 (m, 1 H, 19-H_a), 2.12 (m, 1 H, 19-H_b), 2.79 (m, 2 H, 18-H), 2.95 (s, 3 H, 23-H), 3.28 (dd, $^2J_{4a,4b}$ = 13.8 Hz, $^3J_{4a,3}$ = 6.5 Hz, 1 H, 4-H_a), 3.35 (dd, $^2J_{4b,4a}$ = 13.8 Hz, $^3J_{4b,3}$ = 6.1 Hz, 1 H, 4-H_b), 3.72 (s, 3 H, 1-H), 4.31 (m, 1 H, 20-H), 4.55 (dd, $^3J_{25,NH}$ = 9.3 Hz, $^3J_{25,26}$ = 6.7 Hz, 1 H, 25-H), 5.04–5.11 (m, 3 H, 3-H, 31-H), 5.52 (d, $^3J_{NH,25}$ = 9.3 Hz, 1 H, NH), 7.20–7.35 (m, 7 H, 6-H, 33-H, 34-H, 35-H), 7.46 (dd, $^3J_{12,11}$ = $^3J_{12,13}$ = 7.6 Hz, 2 H, 12-H), 7.57 (t, $^3J_{13,12}$ = 7.4 Hz, 1 H, 13-H), 7.72 (d, $^3J_{7,6}$ = 8.2 Hz, 2 H, 7-H), 7.76 (m, 2 H, 11-H), 7.85 ($^3J_{NH,3}$ = 8.1 Hz, 1 H, NH), 7.95 (s, 1 H, 16-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.3 (q, C-29), 16.0 (q, C-27), 19.5 (q, C-22), 20.0 (q, C-22'), 23.7 (t, C-28), 29.2 (t, C-19), 29.6 (q, C-23), 30.0 (d, C-21), 30.3 (t, C-18),

37.5 (d, C-26), 38.1 (t, C-4), 52.4 (q, C-1), 53.0 (d, C-3), 55.8 (d, C-25), 58.9 (d, C-20), 66.8 (t, C-32), 123.2 (d, C-16), 127.8, 128.0, 128.3, 128.4 (4 d, C-12, C-33, C-33, C-34), 129.2 (d, C-6), 129.9 (d, C-11), 130.3 (d, C-7), 132.3 (d, C-13), 136.2, 136.4 (2 s, C-8, C-32), 137.5 (s, C-10), 141.1 (s, C-5), 148.8 (s, C-15), 156.4 (s, C-30), 160.6 (s, C-14), 170.2 (s, C-17), 171.5 (s, C-2), 173.2 (s, C-24), 196.2 (s, C-9).

minor rotamer (selected signals)

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 1.03 (d, $^{3}J_{22,21}$ = 6.7 Hz, 3 H, 22-H), 2.70 (s, 3 H, 23-H), 3.58 (m, 1 H, 20-H), 4.63 (dd, $^{3}J_{25,NH}$ = 9.5 Hz, $^{3}J_{25,26}$ = 6.6 Hz, 1 H, 25-H), 5.63 (d, $^{3}J_{NH,25}$ = 9.7 Hz, 1 H, NH), 7.91 (s, 1 H, 16-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.3 (q, C-29), 16.2 (q, C-27), 20.3 (q, C-22), 20.4 (q, C-22‘), 23.5 (t, C-28), 27.4 (q, C-23), 30.3 (t, C-19), 31.3 (t, C-18), 37.9 (d, C-26), 55.2 (d, C-25), 62.8 (d, C-14), 66.8 (t, C-32), 132.3 (d, C-16), 132.3 (d, C-13), 137.6 (s, C-10), 141.3 (s, C-5), 160.7 (s, C-14), 171.5 (s, C-2).

optical rotation: $[\alpha]^{21}_{D}$ = –1.2° (c = 1.0, $CHCl_3$)

HRMS (CI)	calculated	found
$C_{42}H_{51}N_4O_5S$ $[M+H]^+$	755.3478	755.3440

elemental analysis:

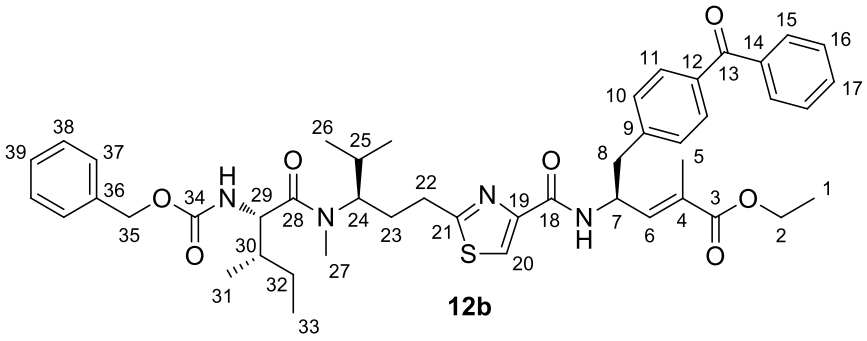
$C_{42}H_{50}N_4O_7S$	calculated	C 66.82	H 6.68	N 7.42
(754.93)	found	C 66.30	H 6.70	N 7.29

(*S*,*E*)-ethyl 5-(4-benzoylphenyl)-4-(2-((*R*)-3-((2*S*,3*S*)-2-(benzyloxycarbonyl-amino)-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methylpent-2-enoate (12b)

HCl in dioxane (3.0 mL, 12.0 mmol, 4 M) was added at 0°C to dipeptide **11b** (182 mg, 0.30 mmol). The solvent was evaporated in vacuum after complete deprotection (30 min, tlc monitoring) and the hydrochloride salt was dried in high vacuum.

N-Deprotected **11b**, Cbz-protected L-isoleucine (90 mg, 0.33 mmol) and BEP (90 mg, 0.33 mmol) were dissolved in dry DCM (3 mL) and diisopropyl ethylamine (160 µL, 0.94 mmol) was added dropwise to this solution 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 DCM and washed with aqueous HCl (1 M), saturated aqueous $NaHCO_3$, water and brine, dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by flash chromatography (SiO_2, hexanes/ethyl acetate = 1:1) to yield tripeptide **12b** (195 mg, 0.26 mmol, 86% over 2 steps) as a white solid and a mixture of rotamers, m.p. 58–60°C.

[tlc: hexanes/ethyl acetate = 1:1, R_f(**12b**) = 0.27]



12b

major rotamer

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.78 (d, $^3J_{26,25}$ = 6.6 Hz, 3 H, 26-H), 0.86 (t, $^3J_{33,32}$ = 7.4 Hz, 3 H, 33-H), 0.96 (d, $^3J_{26',25}$ = 6.5 Hz, 3 H, 26'-H), 0.99 (d, $^3J_{31,30}$ = 6.8 Hz, 3 H, 31-H), 1.13 (m, 1 H, 32-H_a), 1.25 (t, $^3J_{1,2}$ = 7.0 Hz, 3 H, 1-H), 1.59 (m, 1 H, 32-H_b), 1.70 (m, 1 H, 25-H), 1.75 (d, $^4J_{5,7}$ = 1.3 Hz, 3 H, 5-H), 1.77–1.92 (m, 2 H, 23-H_a, 30-H), 2.09 (m, 1 H, 23-H_b), 2.81 (t, $^3J_{22,23}$ = 7.6 Hz, 2 H, 22-H), 2.98 (s, 3 H, 27-H), 3.01 (dd, $^2J_{8a,8b}$ = 13.3 Hz, $^3J_{8a,7}$ = 7.7 Hz, 1 H, 8-H_a), 3.20 (dd, $^2J_{8b,8a}$ = 13.4 Hz, $^3J_{8b,7}$ = 5.8 Hz, 1 H, 8-H_b), 4.16 (q, $^3J_{2,1}$ = 7.1 Hz, 2 H, 2-H), 4.39 (m, 1 H, 24-H), 4.56 (dd, $^3J_{29,NH}$ = 9.4 Hz, $^3J_{29,30}$ = 7.1 Hz, 1 H, 29-H), 5.09 (d, $^2J_{35a,35b}$ = 12.4 Hz, 1 H, 35-H_a), 5.11 (d, $^2J_{35b,35a}$ = 12.4 Hz, 1 H, 35-H_b), 5.20 (m, 1 H, 7-H), 5.50 (d, $^3J_{NH,29}$ = 9.4 Hz, 1 H, NH), 6.73 (dq, $^3J_{6,5}$ = 9.4 Hz, $^4J_{6,5}$ = 1.4 Hz, 1 H, 6-H), 7.22–7.37 (m, 7 H, 10-H, 37-H, 38-H, 39-H), 7.47 (dd, $^3J_{16,17}$ = $^3J_{16,15}$ = 7.6 Hz, 2 H, 16-H), 7.57 (t, $^3J_{17,16}$ =

7.4 Hz, 1 H, 17-H), 7.66 (d, $^{3}J_{NH,7}$ = 8.3 Hz, 1 H, NH), 7.72 (d, $^{3}J_{11,10}$ = 8.2 Hz, 1 H, 11-H), 7.76 (m, 1 H, 15-H), 7.92 (s, 1 H, 20-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.2 (q, C-33), 12.8 (q, C-5), 14.2 (q, C-1), 15.9 (q, C-31), 19.5 (q, C-26), 20.1 (q, C-26‘), 23.9 (t, C-32), 29.4 (t, C-23), 29.6 (q, C-27), 29.9 (t, C-22), 30.2 (d, C-25), 37.6 (d, C-30), 41.0 (t, C-8), 48.7 (d, C-7), 55.8 (d, C-29), 58.6 (d, C-24), 60.8 (t, C-2), 66.8 (t, C-35), 122.7 (d, C-20), 127.8, 128.0, 128.2, 128.4 (4 d, C-16, C-37, C-38, C-38), 129.4 (d, C-10), 129.9 (d, C-15), 130.3 (d, C-11), 130.7 (s, C-4), 132.3 (d, C-17), 136.0 (s, C-12), 136.4 (s, C-36), 137.7 (s, C-14), 138.6 (d, C-6), 141.9 (s, C-9), 149.3 (s, C-19), 156.5 (s, C-34), 160.4 (s, C-18), 167.7 (s, C-21), 169.8 (s, C-28), 173.3 (s, C-3), 196.3 (s, C-13).

minor rotamer (selected signals)

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.91 (d, $^{3}J_{26,25}$ = 6.7 Hz, 3 H, 26-H), 1.02 (d, $^{3}J_{26',25}$ = 6.5 Hz, 3 H, 26‘-H), 1.29 (t, $^{3}J_{1,2}$ = 7.6 Hz, 3 H, 1-H), 2.74 (s, 3 H, 27-H), 2.89 (m, 2 H, 22-H), 3.52 (m, 1 H, 24-H), 4.19 (q, $^{3}J_{2,1}$ = 7.0 Hz, 2 H, 2-H), 4.76 (dd, $^{3}J_{29,NH}$ = 9.6 Hz, $^{3}J_{29,30}$ = 5.8 Hz, 1 H, 29-H), 5.60 (d, $^{3}J_{NH,29}$ = 9.6 Hz, 1 H, NH), 7.82 (d, $^{3}J_{NH,7}$ = 8.3 Hz, 1 H, NH), 7.93 (s, 1 H, 20-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.3 (q, C-33), 12.8 (q, C-5), 14.2 (q, C-1), 16.3 (q, C-31), 20.3 (q, C-26), 20.4 (q, C-26‘), 23.3 (t, C-32), 27.6 (q, C-27), 37.9 (d, C-30), 55.4 (d, C-29), 60.8 (t, C-2), 66.9 (t, C-35), 123.2 (d, C-20), 128.2 (d, C-16), 132.3 (d, C-17), 149.4 (s, C-19), 167.6 (s, C-21), 170.2 (s, C-28).

optical rotation: $[\alpha]^{21}_{D}$ = +11.2° (c = 1.0, $CHCl_3$)

HRMS (CI)	calculated	found
$C_{46}H_{58}N_4O_7S$ $[M+2H]^{2+}$	810.4026	810.4061

elemental analysis:

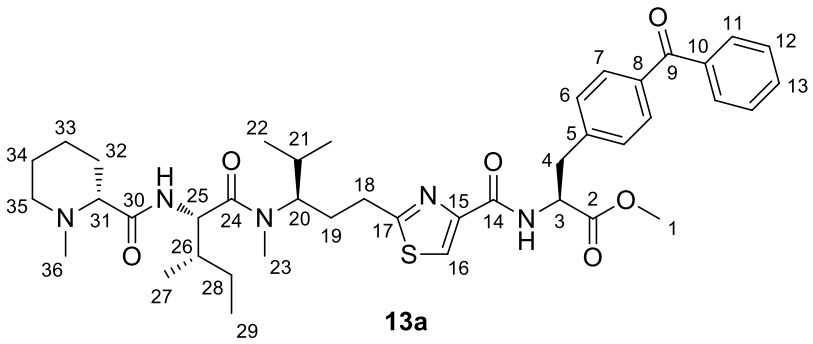
$C_{46}H_{56}N_4O_7S$	calculated	C 68.29	H 6.98	N 6.93
(809.02)	found	C 67.73	H 7.07	N 6.73

(*S*)-methyl 3-(4-benzoylphenyl)-2-(2-((*R*)-3-((2*S*,3*S*)-*N*,3-dimethyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)propanoate (13a)

HBr in HOAc (120 µL, 0.70 mmol, 33 wt.-%) was added at room temperature to tripeptide **12a** (52 mg, 0.30 mmol). After complete deprotection (1 h, tlc monitoring) the reaction mixture was diluted with diethyl ether (5 mL). The hydrobromide salt was filtered off, washed with diethyl ether (3 x 5 mL) and treated with saturated aqueous $NaHCO_3$ (5 mL). The free amine was extracted with DCM and the solvent was removed in vacuum to get the crude amine as a colourless oil.

A solution of *N*-methyl pipecolic acid (21 mg, 0.15 mmol), pentafluorophenol (31 mg, 0.17 mmol) and DCC (35 mg, 0.17 mmol) in dry DCM (2 mL) was stirred at room temperature for 1 day. The urea was filtered off and the filtrate was stirred with *N*-deprotected **12a** for 20 h. The reaction mixture was diluted with DCM and washed with saturated aqueous $NaHCO_3$, water and brine, dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by flash chromatography (SiO_2, DCM/MeOH = 98:2, 95:5) to yield tetrapeptide **13a** (38 mg, 51 µmol, 74% over 2 steps) as a white solid and a mixture of rotamers, m.p. 64–66°C.

[tlc: DCM/MeOH = 95:5, R_f(**13a**) = 0.21]



13a

major rotamer

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.74 (d, $^3J_{22,21}$ = 6.6 Hz, 3 H, 22-H), 0.88 (t, $^3J_{29,28}$ = 7.4 Hz, 3 H, 29-H), 0.94 (d, $^3J_{22',21}$ = 6.5 Hz, 3 H, 22'-H), 0.96 (d, $^3J_{27,26}$ = 6.7 Hz, 3 H, 27-H), 1.10–1.24 (m, 2 H, 28-H_a, 33-H_{ax}), 1.34 (m, 1 H, 32-H_{ax}), 1.45–1.73 (m, 5 H, 21-H, 28-H_b, 33-H_{eq}, 34-H), 1.77–1.90 (m, 3 H, 19-H_a, 26-H, 32-H_{eq}), 1.99 (ddd, $^2J_{35ax,35eq}$ = 11.7 Hz, $^3J_{35ax,34ax}$ = 11.7 Hz, $^3J_{35ax,34eq}$ = 2.2 Hz, 1 H, 35-H_{ax}), 2.14 (m, 1 H, 19-H_b), 2.21 (s, 3 H, 36-H), 2.46 (dd, $^3J_{31a,32eq}$ = 10.9 Hz, $^3J_{31,32ax}$ = 2.7 Hz, 1 H, 31-H), 2.77–2.92 (m, 3 H, 18-H, 35-H_{eq}), 2.98 (s, 3 H, 23-H), 3.28 (dd, $^2J_{4a,4b}$ = 13.8 Hz, $^3J_{4a,3}$ = 6.4 Hz, 1 H, 4-H_a), 3.35 (dd, $^2J_{4b,4a}$ = 13.8 Hz, $^3J_{4b,3}$ = 6.0 Hz, 1 H, 4-H_b), 3.74 (s, 3 H, 1-H), 4.33 (m, 1 H, 20-H), 4.76 (dd, $^3J_{25,NH}$ = $^3J_{25,26}$ = 8.8 Hz, 1 H, 25-H), 5.08 (m, 1 H, 3-H), 7.02 (d, $^3J_{NH,25}$ = 9.4 Hz, 1 H, NH), 7.30 (d, $^3J_{6,7}$ = 8.0 Hz,

2 H, 6-H), 7.46 (dd, $^3J_{12,11}$ = $^3J_{12,13}$ = 7.6 Hz, 2 H, 12-H), 7.57 (t, $^3J_{13,12}$ = 7.3 Hz, 1 H, 13-H), 7.72 (d, $^3J_{7,6}$ = 8.1 Hz, 2 H, 7-H), 7.75 (d, $^3J_{11,12}$ = 7.9 Hz, 2 H, 11-H), 7.85 ($^3J_{NH,3}$ = 8.1 Hz, 1 H, NH), 7.94 (s, 1 H, 16-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 10.9 (q, C-29), 15.9 (q, C-27), 19.5 (q, C-22), 20.1 (q, C-22‘), 23.2 (t, C-28), 24.5 (t, C-32), 25.0 (t, C-34), 29.2 (t, C-19), 29.6 (q, C-23), 30.0 (d, C-21), 30.2 (t, C-18), 30.4 (t, C-32), 37.0 (d, C-26), 38.1 (t, C-4), 44.8 (q, C-36), 52.4 (q, C-1), 53.0 (2 d, C-3, C-25), 53.0 (t, C-35), 58.7 (d, C-20), 69.6 (d, C-31), 123.2 (d, C-16), 128.2 (d, C-12), 129.2 (d, C-6), 129.9 (d, C-11), 130.3 (d, C-7), 132.3 (d, C-13), 136.3 (s, C-8), 137.6 (s, C-10), 141.1 (s, C-5), 148.9 (s, C-15), 160.6 (s, C-14), 170.3 (s, C-17), 171.5 (s, C-2), 173.2 (s, C-24), 174.2 (s, C-30), 196.2 (s, C-9).

minor rotamer (selected signals)

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.96 (d, $^3J_{27,26}$ = 6.4 Hz, 3 H, 27-H), 2.75 (s, 3 H, 23-H), 3.64 (m, 1 H, 20-H), 4.96 (dd, $^3J_{25,NH}$ = 9.4 Hz, $^3J_{25,26}$ = 6.3 Hz, 1 H, 25-H), 7.16 (d, $^3J_{NH,25}$ = 9.7 Hz, 1 H, NH), 7.91 (s, 2 H, 16-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.4 (q, C-29), 16.5 (q, C-27), 20.3 (q, C-22), 20.6 (q, C-22‘), 27.3 (q, C-23), 38.4 (d, C-26), 62.8 (d, C-20), 123.3 (d, C-16), 141.2 (s, C-5), 170.1 (s, C-17), 172.9 (s, C-24).

optical rotation: $[\alpha]^{21}_D$ = +11.8° (c = 1.0, $CHCl_3$)

HRMS (CI)	calculated	found
$C_{41}H_{56}N_5O_6S$ $[M+H]^+$	746.3951	746.3964

elemental analysis:

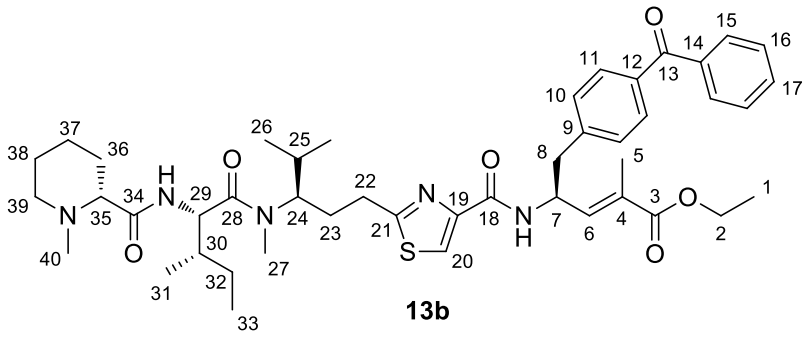
$C_{41}H_{55}N_5O_6S$	calculated	C 66.01	H 7.43	N 9.39
(745.97)	found	C 65.90	H 7.45	N 9.41

(*S*,*E*)-ethyl 5-(4-benzoylphenyl)-4-(2-((*R*)-3-((2*S*,3*S*)-*N*,3-dimethyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methylpent-2-enoate (13b)

HBr in HOAc (0.70 mL, 4.05 mmol, 33 wt.-%) was added at room temperature to tripeptide **12b** (319 mg, 0.39 mmol). After complete deprotection (1 h, tlc monitoring) the reaction mixture was diluted with diethyl ether (10 mL). The hydrobromide salt was filtered off, washed with diethyl ether (3 x 10 mL) and treated with saturated aqueous $NaHCO_3$ (5 mL). The free amine was extracted with DCM and the solvent was removed in vacuum to get the crude amine as a colourless oil.

A solution of *N*-methyl pipecolic acid (112 mg, 0.78 mmol), pentafluorophenol (158 mg, 0.86 mmol) and DCC (177 mg, 0.86 mmol) in dry DCM (8 mL) was stirred at room temperature for 1 day. The urea was filtered off and the filtrate was stirred with *N*-deprotected **12b** for 20 h. The reaction mixture was diluted with DCM and washed with saturated aqueous $NaHCO_3$, water and brine, dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by flash chromatography (SiO_2, DCM/MeOH = 98:2, 95:5) to yield tetrapeptide **13b** (196 mg, 0.24 mmol, 66% over 2 steps) as a white solid and a mixture of rotamers, m.p. 68–70°C.

[tlc: DCM/MeOH = 95:5, R_f(**13b**) = 0.18]



13b

major rotamer

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.79 (d, $^3J_{26,25}$ = 6.6 Hz, 3 H, 26-H), 0.90 (t, $^3J_{33,32}$ = 7.4 Hz, 3 H, 33-H), 0.98 (d, $^3J_{26',25}$ = 6.6 Hz, 3 H, 26'-H), 1.00 (d, $^3J_{31,30}$ = 6.8 Hz, 3 H, 31-H), 1.13–1.22 (m, 1 H, 32-H_a, 37-H_{ax}), 1.29 (t, $^3J_{1,2}$ = 7.1 Hz, 3 H, 1-H), 1.39 (m, 1 H, 36-H_{ax}), 1.51–1.75 (m, 5 H, 25-H, 32-H_b, 37-H_{eq}, 38-H), 1.78 (s, 3 H, 5-H), 1.82–1.92 (m, 3 H, 23-H_a, 30-H, 36-H_{eq}), 2.05 (m, 1 H, 39-H_{ax}), 2.13 (m, 1 H, 23-H_b), 2.24 (s, 3 H, 40-H), 2.50 (m, 1 H, 35-H), 2.85 (t, $^3J_{22,23}$ = 8.0 Hz, 2 H, 22-H), 2.90 (m, 1 H, 39-H_{eq}), 3.03 (s, 3 H, 27-H), 3.04 (dd, $^2J_{8a,8b}$ = 13.2 Hz, $^3J_{8a,7}$ = 7.8 Hz, 1 H, 8-H_a), 3.25 (dd, $^2J_{8b,8a}$ = 13.4 Hz, $^3J_{8b,7}$ = 6.0 Hz, 1 H, 8-H_b), 4.20 (q, $^3J_{2,1}$ = 7.1 Hz, 2 H, 2-H), 4.23 (m 1 H, 24-H), 4.81 (dd, $^3J_{29,NH}$ = 9.3 Hz, $^3J_{29,30}$ = 8.2 Hz, 1 H, 29-H), 5.23 (m, 1 H, 7-H), 6.74 (d, $^3J_{6,7}$ = 9.3 Hz, 1 H, 6-H), 7.07 (d, $^3J_{NH,29}$ = 9.2 Hz, 1 H, NH), 7.37

(d, $^{3}J_{10,11}$ = 8.2 Hz, 2 H, 10-H), 7.49 (dd, $^{3}J_{16,15}$ = $^{3}J_{16,17}$ = 7.6 Hz, 2 H, 16-H), 7.59 (tt, $^{3}J_{17,16}$ = 7.4 Hz, $^{4}J_{17,15}$ = 1.3 Hz, 1 H, 17-H), 7.68 (d, $^{3}J_{NH,7}$ = 8.4 Hz, 1 H, NH), 7.75 (d, $^{3}J_{11,10}$ = 8.2 Hz, 2 H, 11-H), 7.77 (m, 2 H, 15-H), 7.94 (s, 1 H, 20-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 10.9 (q, C-33), 12.8 (q, C-5), 14.2 (q, C-1), 15.9 (q, C-31), 19.6 (q, C-26), 20.1 (q, C-26‘), 23.2 (t, C-32), 24.6 (t, C-37), 25.0 (t, C-38), 29.4 (t, C-23), 29.6 (q, C-27), 30.0 (t, C-22), 30.2 (d, C-25), 30.4 (t, C-36), 37.1 (d, C-30), 41.0 (t, C-8), 44.8 (q, C-40), 48.6 (d, C-7), 53.0 (d, C-29), 55.4 (t, C-39), 60.8 (t, C-2), 62.7 (d, C-24), 69.6 (d, C-35), 122.7 (d, C-20), 128.2 (d, C-16), 129.4 (d, C-10), 129.9 (d, C-15), 130.3 (d, C-11), 130.5 (s, C-4), 132.3 (d, C-17), 135.9 (s, C-12), 137.6 (s, C-14), 138.7 (d, C-6), 141.9 (s, C-9), 149.3 (s, C-19), 160.4 (s, C-18), 167.7 (s, C-21), 169.9 (s, C-28), 173.2 (s, C-3), 174.2 (s, C-34), 196.3 (s, C-13).

minor rotamer (selected signals)

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.91 (t, $^{3}J_{33,32}$ = 7.1 Hz, 3 H, 33-H), 1.06 (d, $^{3}J_{26,25}$ = 6.5 Hz, 3 H, 26-H), 2.79 (s, 3 H, 27-H), 3.63 (m 1 H, 24-H), 5.08 (dd, $^{3}J_{29,NH}$ = 9.6 Hz, $^{3}J_{29,30}$ = 5.7 Hz, 1 H, 29-H), 7.23 (d, $^{3}J_{NH,29}$ = 9.6 Hz, 1 H, NH), 7.93 (s, 1 H, 20-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.4 (q, C-33), 16.6 (q, C-31), 20.4 (q, C-26), 20.5 (q, C-26‘), 27.4 (q, C-27), 38.4 (d, C-30), 44.9 (q, C-40), 58.4 (d, C-24), 60.6 (t, C-2), 123.0 (d, C-22), 135.9 (d, s, C-12), 137.6 (s, C-14), 142.0 (s, C-9), 170.0 (s, C-28), 172.9 (s, C-3).

optical rotation: $[\alpha]^{21}_{D}$ = +27.5° (c = 0.8, $CHCl_3$)

HRMS (CI)	calculated	found
$C_{31}H_{50}N_5O_5S$ $[M–C_{14}H_{11}O+H]^+$	604.3533	604.3530

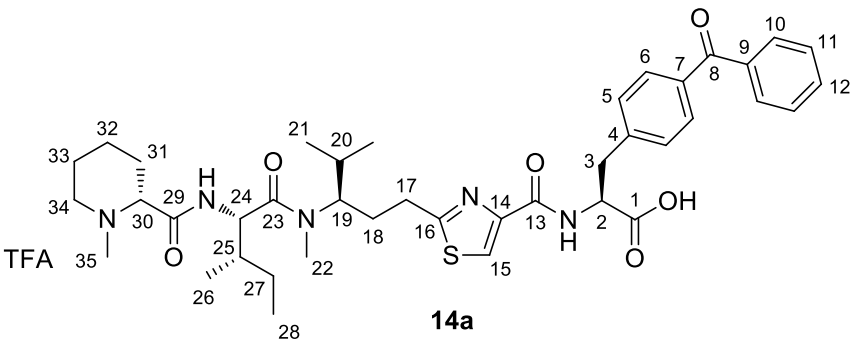
elemental analysis

$C_{45}H_{61}N_5O_6S$	calculated	C 67.56	H 7.68	N 8.44
(800.06)	found	C 66.96	H 7.94	N 8.44

(*S*)-3-(4-benzoylphenyl)-2-(2-((*R*)-3-((2*S*,3*S*)-*N*,3-dimethyl-2-((*R*)-1-methyl-piperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carbox-amido)propanoic acid-TFA-salt (14a)

A mixture of *N*-methylated tetrapeptide **13a** (50 mg, 67 µmol) and 1 M NaOH (140 µL, 140 µmol) in dioxane (1 mL) was stirred at 80°C until complete saponification occurred (6 h). The solvent was removed in vacuum 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 vacuum. Purification by flash chromatography (DCM/ MeOH, 9:1) provided the TFA salt of **14a** (52 mg, 61 µmol, 92%) as a white solid and a mixture of rotamers; m.p. 186–188°C.

[tlc: DCM/MeOH = 9:1, R_f(**14a**) = 0.11]



14a

major rotamer

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.74 (d, $^3J_{21,20}$ = 6.6 Hz, 3 H, 21-H), 0.88 (t, $^3J_{28,27}$ = 7.4 Hz, 3 H, 28-H), 0.91 (d, $^3J_{21',20}$ = 6.3 Hz, 3 H, 21'-H), 0.97 (d, $^3J_{26,25}$ = 6.7 Hz, 3 H, 26-H), 1.18 (m, 1 H, 27-H_a), 1.52 (m, 1 H, 32-H_{ax}), 1.62 (m, 1 H, 27-H_b), 1.68–1.78 (m, 3 H, 20-H, 32-H_{eq}, 33-H_{ax}), 1.80–1.95 (4 H, 18-H_a, 25-H, 31-H_{ax}, 33-H_{eq}), 2.01 (m, 1 H, 31-H_{eq}), 2.17 (m, 1 H, 18-H_b), 2.54 (s, 3 H, 35-H), 2.73 (m, 1 H, 34-H_{ax}), 2.85 (m, 2 H, 17-H), 3.04 (s, 3 H, 22-H), 3.25–3.30 (m, 2 H, 3-H_a, 34-H_{eq}), 3.38 (m, 1 H, 30-H), 3.44 (dd, $^2J_{3b,3a}$ = 13.6 Hz, $^3J_{3b,2}$ = 5.1 Hz, 1 H, 3-H_b), 4.17 (bs, 1 H, 19-H), 4.72 (d, $^3J_{24,NH}$ = 7.9 Hz, 1 H, 24-H), 4.77 (t, $^3J_{2,3}$ = 5.7 Hz, 1 H, 2-H), 7.40 (d, $^3J_{5,6}$ = 8.1 Hz, 2 H, 5-H), 7.50 (dd, $^3J_{11,10}$ = $^3J_{11,12}$ = 7.6 Hz, 2 H, 11-H), 7.61–7.64 (m, 3 H, 6-H, 12-H), 7.71 (d, $^3J_{10,11}$ = 7.7 Hz, 2 H, 10-H), 8.04 (s, 1 H, 15-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.4 (q, C-28), 16.3 (q, C-26), 20.3 (q, C-21), 20.6 (q, C-21'), 23.0 (t, C-27), 24.8 (t, C-32), 25.5 (t, C-33), 30.1 (t, C-18), 30.5 (q, C-22), 30.7 (t, C-31), 31.1 (t, d, C-17, C-20), 37.6 (d, C-25), 39.1 (t, C-3), 43.5 (q, C-35), 55.8 (d, C-24), 56.4 (t, C-34), 56.8 (d, C-19), 69.0 (d, C-30), 124.5 (d, C-15), 129.5 (d, C-11), 130.9 (2 d, C-5, C-10), 131.1 (d, C-6), 133.7 (d, C-12), 136.9 (s, C-7),

139.0 (s, C-9), 145.0 (s, C-4), 150.4 (s, C-14), 162.7 (s, C-13), 171.4 (s, C-16), 172.1 (s, C-23), 174.5 (s, C-29), 177.9 (s, C-1), 198.2 (s, C-8).

minor rotamer (selected signals)

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 1.06 (d, $^{3}J_{26,25}$ = 6.4 Hz, 3 H, 26-H), 2.50 (s, 3 H, 35-H), 2.61 (s, 3 H, 22-H), 3.68 (m, 1 H, 19-H), 4.72 (d, $^{3}J_{24,NH}$ = 5.4 Hz, 1 H, 24-H), 7.97 (s, 1 H, 16-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.8 (q, C-28), 16.7 (q, C-26), 21.0 (q, C-21), 28.3 (q, C-22), 38.0 (d, C-25), 39.3 (t, C-3), 43.5 (q, C-35), 150.5 (s, C-14).

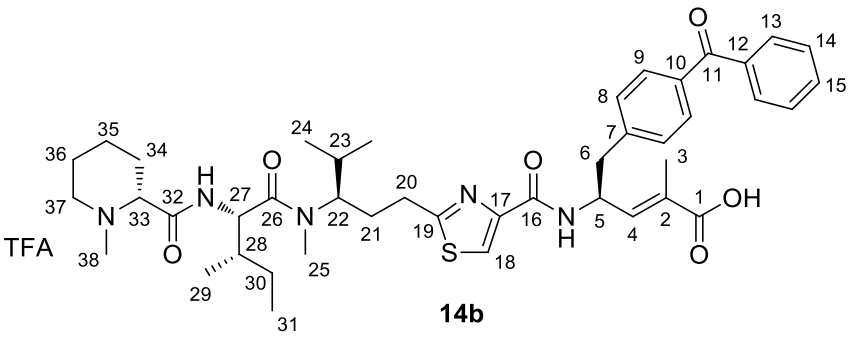
optical rotation: $[\alpha]^{21}_{D}$ = +7.9° (c = 0.6, MeOH)

HRMS (ESI)	calculated	found
$C_{40}H_{54}N_5O_6S$ $[M+H]^+$	732.3795	732.3769

(*S*,*E*)-5-(4-benzoylphenyl)-4-(2-((*R*)-3-((2*S*,3*S*)-*N*,3-dimethyl-2-((*R*)-1-methyl-piperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carbox-amido)-2-methylpent-2-enoic acid-TFA-salt (14b)

A mixture of *N*-methylated tetrapeptide **13b** (50 mg, 62 µmol) and 1 M NaOH (180 µL, 180 µmol) in dioxane (1 mL) was stirred at 80°C until complete saponification occurred (7 h). The solvent was removed in vacuum 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 vacuum. Purification by flash chromatography (DCM/ MeOH, 9:1) provided the TFA salt of **14b** (53 mg, 60 µmol, 97%) as a white solid and a mixture of rotamers; m.p. 158–160°C.

[tlc: DCM/MeOH = 9:1, R_f(**14b**) = 0.27]



14b

major rotamer

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.65 (d, $^3J_{24,23}$ = 6.6 Hz, 3 H, 24-H), 0.80 (t, $^3J_{31,30}$ = 7.4 Hz, 3 H, 31-H), 0.83 (d, $^3J_{24',23}$ = 6.5 Hz, 3 H, 24'-H), 0.90 (d, $^3J_{29,28}$ = 6.8 Hz, 3 H, 29-H), 1.13 (m, 1 H, 30-H_a), 1.38 (m, 1 H, 35-H_{ax}), 1.48 (m, 1 H, 30-H_b), 1.57–1.76 (m, 8 H, 3-H, 23-H, 34-H_{ax}, 35-H_{eq}, 36-H), 1.79–1.92 (m, 3 H, 21-H_a, 28-H, 34-H_{eq}), 2.03 (m, 1 H, 21-H_b), 2.45 (s, 3 H, 38-H), 2.66 (ddd, $^2J_{37ax,37eq}$ = 12.5 Hz, $^3J_{37ax,36ax}$ = 12.5 Hz, $^3J_{37ax,36eq}$ = 2.9 Hz, 1 H, 37-H_{ax}), 2.75 (m, 1 H, 20-H_a), 2.85 (m, 1 H, 20-H_b), 2.94 (dd, $^2J_{6a,6b}$ = 13.0 Hz, $^3J_{6a,5}$ = 7.5 Hz, 1 H, 6-H_a), 2.98 (s, 3 H, 25-H), 3.09 (dd, $^2J_{6b,6a}$ = 13.3 Hz, $^3J_{6b,5}$ = 6.9 Hz, 1 H, 6-H_b), 3.19 (m, 1 H, 37-H_{eq}), 3.33 (dd, $^2J_{33,34eq}$ = 11.5 Hz, $^3J_{33,34ax}$ = 2.4 Hz, 1 H, 33-H), 4.18 (m, 1 H, 22-H), 4.61 (d, $^3J_{27,28}$ = 8.6 Hz, 1 H, 27-H), 5.05 (m, 1 H, 5-H), 6.60 (dq, $^3J_{4,5}$ = 9.2 Hz, $^4J_{4,3}$ = 1.0 Hz, 1 H, 4-H), 7.31 (d, $^3J_{8,9}$ = 8.1 Hz, 2 H, 8-H), 7.39 (dd, $^3J_{14,15}$ = $^3J_{14,13}$ = 7.6 Hz, 2 H, 14-H), 7.51 (t, $^3J_{15,14}$ = 7.5 Hz, 1 H, 15-H), 7.56 (d, $^3J_{9,8}$ = 8.2 Hz, 2 H, 9-H), 7.60 (m, 2 H, 13-H), 7.91 (s, 1 H, 18-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.2 (q, C-31), 13.9 (q, C-29), 16.0 (q, C-3), 20.3 (q, C-24), 20.6 (q, C-24'), 23.0 (t, C-30), 24.7 (t, C-35), 25.7 (t, C-36), 30.3 (q, C-25), 30.5 (t, C-21), 30.6 (t, C-34), 31.0 (t, C-20), 31.4 (d, C-23), 37.7 (d, C-28), 41.9 (t,

C-6), 43.4 (q, C-38), 50.8 (d, C-5), 55.6 (d, C-27), 56.3 (t, C-37), 60.2 (d, C-22), 69.0 (d, C-33), 118.2 (q, $^{2}J_{C,F}$ = 291 Hz, $\underline{C}F_3COOH$), 124.5 (d, C-18), 129.5 (d, C-14), 130.9 (2 d, C-8, C-13), 130.9 (d, C-9), 133.7 (d, C-15), 137.0, 137.1 (d, s, C-4, C-10), 139.0 (s, C-12), 144.5 (s, C-7), 150.4 (s, C-17), 162.7 (s, C-16), 163.2 (q, $^{3}J_{C,F}$ = 34.6 Hz, $CF_3\underline{C}OOH$), 171.2 (s, C-19), 171.9 (s, C-26), 174.8 (s, C-32), 175.6 (s, C-19), 198.2 (s, C-11).

minor rotamer

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.98 (d, $^{3}J_{24,23}$ = 6.5 Hz, 3 H, 24-H), 2.35 (s, 3 H, 38-H), 2.55 (s, 3 H, 25-H), 4.67 (d, $^{3}J_{27,28}$ = 8.9 Hz, 1 H, 27-H), 7.88 (s, 1 H, 18-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.9 (q, C-31), 16.5 (q, C-3), 20.7 (q, C-24), 20.9 (q, C-24‘), 28.3 (q, C-25), 38.5 (d, C-28), 43.6 (q, C-38), 64.7 (d, C-22).

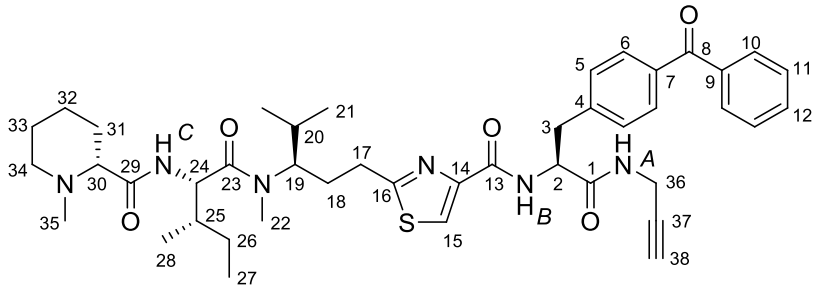
optical rotation: $[\alpha]^{21}_{D}$ = –4.5° (c = 0.9, MeOH)

HRMS (ESI)	calculated	found
$C_{43}H_{58}N_5O_6S$ $[M+H]^+$	772.4108	772.4082

_N_-((_S_)-3-(4-Benzoylphenyl)-1-oxo-1-(prop-2-ynylamino)propan-2-yl)-2-((_R_)-3-((2_S_,3_S_)-_N_,3-dimethyl-2-((_R_)-1-methyl-piperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carbox-amide (4)

At –20°C isobutylchloroformiate (3 µl, 23.1 µmol) was added dropwise to a solution of benzophenon pretubulysin derivative **14a** (16.7 mg, 19.7 µmol) and *N*-methylmorpholine (9 µl, 81.9 µmol) in dry THF/DMF (2:1, 600 µl). After 10 min propargylamine hydrochloride (3 mg, 31.1 µmol) was added and the reaction mixture was stirred overnight. The solvents were evaporated and the residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$ solution. The aqueous layer was extracted twice with ethyl acetate and the combined organic layers were dried over Na_2SO_4. The crude product was purified by flash chromatography (DCM/MeOH 98:2 – 9:1) to yield the propargylamide ***4*** (8 mg, 10.4 µmol, 53 %) as a pale yellow solid and a mixture of rotamers; m.p.85 °C.

[tlc: DCM/MeOH = 9:1, R_f(**4**) = 0.18]



major rotamer

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.74 (d, $^3J_{21,20}$ = 6.6 Hz, 3 H, 21-H), 0.86 (t, $^3J_{27,26}$ = 7.4 Hz, 3 H, 27-H), 0.92 (d, $^3J_{21',20}$ = 6.6 Hz, 3 H, 21'-H), 0.97 (d, $^3J_{28,25}$ = 6.7 Hz, 3 H, 28-H), 1.08-1.70 (m, 9 H, 20-H, 26-H, 31-H_{ax}, 32-H, 33-H), 1.74-1.91 (m, 3 H, 18-H_a, 25-H, 31-H_{eq}), 1.93-2.13 (m, 2 H, 18-H_b, 34-H_{ax}), 2.17 (dd, $^4J_{38,36a}$ = $^4J_{38,36b}$ = 2.5 Hz, 1 H, 38-H), 2.20 (s, 3 H, 35-H), 2.47 (dd, $^3J_{30,31ax}$ = 11.0 Hz, $^3J_{30,31eq}$ = 3.0 Hz, 1 H, 30-H), 2.72-2.99 (m, 3 H, 17-H, 34-H_{eq}), 3.00 (s, 3 H, 22-H), 3.22 (dd, $^2J_{3a,3b}$ = 13.9 Hz, $^3J_{3a,2}$ = 7.5 Hz, 1 H, 3-H_a), 3.38 (dd, $^2J_{3b,3a}$ = 13.9 Hz, $^3J_{3b,2}$ = 6.8 Hz, 1 H, 3-H_b), 3.95 (ddd, $^2J_{36a,36b}$ = 17.5 Hz, $^3J_{36a,NH\ A}$ = 5.1 Hz, $^4J_{36a,38}$ = 2.5 Hz, 1 H, 36-H_a), 4.04 (ddd, $^2J_{36b,36a}$ = 17.5 Hz, $^3J_{36b,NH\ A}$ = 5.6 Hz, $^4J_{36b,38}$ = 2.6 Hz, 1 H, 36-H_a), 4.32 (bs, 1 H, 19-H), 4.76 (dd, $^3J_{24,NH\ C} \approx {}^3J_{24,25} \approx$ 8.9 Hz, 1 H, 24-H), 4.90 (ddd, $^3J_{2,NH\ B} \approx {}^3J_{2,3a} \approx {}^3J_{2,3b} \approx$7.7 Hz, 1 H, 2-H), 6.63 (dd, $^3J_{NH\ A,36a} \approx {}^3J_{NH\ A,36b} \approx$ 5.3 Hz, 1 H, NH_A), 7.01 (d, $^3J_{NH\ C,24}$ = 9.6 Hz, 1 H, NH_C), 7.37 (d, $^3J_{5,6}$ = 8.2 Hz, 2 H, 5-H), 7.44 (dd, $^3J_{11,10} \approx {}^3J_{11,12} \approx$ 7.5 Hz, 2 H, 11-H), 7.56 (tt, $^3J_{12,11}$ = 7.4 Hz, $^4J_{12,10}$ = 1.3 Hz, 1 H, 12-H), 7.71 (d, $^3J_{6,5}$ = 8.3 Hz,

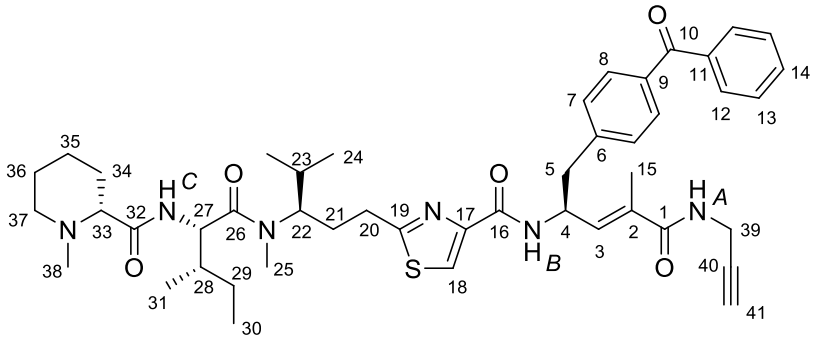
2 H, 6-H), 7.74 (dd, $^{3}J_{10,11}$ = 8.3 Hz, $^{4}J_{10,12}$ = 1.3 Hz, 2 H, 10-H), 7.92 (s, 1 H, 15-H), 7.93 (m, 1 H, NH_B). **^{13}C-NMR** ($CDCl_3$, 100 MHz, selected signals, according to HSQC): δ = 10.9 (q, C-27), 15.9 (q, C-28), 19.6 (q, C-21), 20.1 (q, C-21'), 23.3 (t, C-26), 24.7 (t, C-32), 29.2 (t, C-36), 29.4 (t, C-18), 30.0 (t, C-17), 30.2 (d, C-20), 37.1 (d, C-25), 38.1 (t, C-3), 44.9 (q, C-35), 53.4 (d, C-24), 54.2 (d, C-2), 55.4 (t, C-34), 69.7 (d, C-30), 71.7 (d, C-38), 123.5 (d, C-15), 128.3 (d, C-11), 129.2 (d, C-5), 130.0 (d, C-10), 130.5 (d, C-6), 132.4 (d, C-12), 170.2 (s, C-16).

HRMS (CI)	calculated	found
$C_{43}H_{57}N_6O_5S$ $[M+H]^+$	769.4111	769.4073

_N_-((_S_,_E_)-5-(4-Benzoylphenyl)-1-oxo-1-(prop-2-ynylamino)pent-2-en-4-yl)-2-((_R_)-3-((2_S_,3_S_)-_N_,3-dimethyl-2-((_R_)-1-methyl-piperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carbox-amide (5)

At –20°C isobutylchloroformiate (3 µl, 23.1 µmol) was added dropwise to a solution of benzophenon pretubulysin derivative **14b** (17.4 mg, 19.6 µmol) and *N*-methyl-morpholine (9 µl, 81.9 µmol) in dry THF (400 µl). After 10 min propargylamine hydrochloride (3 mg, 31.1 µmol) was added and the reaction mixture was stirred overnight. The solvent was evaporated and the residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$ solution. The aqueous layer was extracted twice with ethyl acetate and the combined organic layers were dried over Na_2SO_4. The crude product was purified by flash chromatography (DCM/MeOH 97:3 – 9:1) to yield the propargylamide ***5*** (11 mg, 13.6 µmol, 69 %) as a colorless glassy solid and a mixture of rotamers; m.p.82 °C.

[tlc: DCM/MeOH = 9:1, R_f(**5**) = 0.26]



major rotamer

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.75 (d, $^3J_{24,23}$ = 6.6 Hz, 3 H, 24-H), 0.86 (t, $^3J_{30,29}$ = 7.4 Hz, 3 H, 30-H), 0.93 (d, $^3J_{24',23}$ = 6.5 Hz, 3 H, 24'-H), 0.97 (d, $^3J_{31,28}$ = 6.7 Hz, 3 H, 31-H), 1.15 (m, 1 H, 29-H_a), 1.37 (m, 1 H, 34-H_{ax}), 1.42-1.93 (m, 12 H, 15-H, 21-H_a, 23-H, 28-H, 29-H_b, 34-H_{eq}, 35-H, 36-H), 1.98 (ddd, $^2J_{37ax,37eq} \approx {}^3J_{37ax,36ax} \approx$ 11.6 Hz, $^3J_{37ax,36eq}$ = 2.5 Hz, 1 H, 37-H_{ax}), 2.08 (m, 1 H, 21-H_b), 2.20 (s, 3 H, 38-H), 2.20 (m, 1 H, 41-H), 2.48 (dd, $^3J_{33,34ax}$ = 10.8 Hz, $^3J_{33,34eq}$ = 2.4 Hz, 1 H, 33-H), 2.78-2.89 (m, 3 H, 20-H, 37-H_{eq}), 2.99 (m, 1 H, 5-H_a), 3.00 (3 H, 25-H), 3.23 (dd, $^2J_{5b,5a}$ = 13.3 Hz, $^3J_{5b,4}$ = 5.8 Hz, 1-H, 5-H_b), 4.06 (dd, $^3J_{39,NH\ A}$ = 5.2 Hz, $^4J_{39,41}$ = 2.5 Hz, 1 H, 39-H), 4.35 (bs, 1 H, 22-H), 4.77 (dd, $^3J_{27,NH\ C}$ = 9.3 Hz, $^3J_{27,28}$ = 8.3 Hz, 1 H, 27-H), 5.15 (m, 1 H, 4-H), 5.95 (t, $^3J_{NH\ A,39}$ = 4.9 Hz, 1 H, NH_A), 6.47 (d, $^3J_{3,4}$ = 9.6 Hz, 1 H, 3-H), 7.06 (d, $^3J_{NH\ C,27}$ = 9.4 Hz, 1 H, NH_C), 7.34 (d, $^3J_{7,8}$ = 8.2 Hz, 2 H, 7-H), 7.45 (dd,

$^{3}J_{13,12} \approx {}^{3}J_{13,14} \approx 7.5$ Hz, 2 H, 13-H), 7.56 (tt, $^{3}J_{14,13} = 7.4$ Hz, $^{4}J_{14,12} = 1.2$ Hz, 1 H, 14-H), 7.67 (d, $^{3}J_{NH\,B,4} = 8.2$ Hz, 1 H, NH_B), 7.72 (d, $^{3}J_{8,7} = 8.2$ Hz, 2 H, 8-H), 7.74 (m, 2 H, 12-H), 7.90 (s, 1 H, 18-H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.0 (q, C-30), 13.1 (q, C-15), 15.9 (q, C-31), 19.7 (q, C-24), 20.1 (q, C-24'), 23.3 (t, C-29), 24.6 (t, C-35), 25.1 (t, C-36), 29.5 (tq, C-21, C-25), 29.6 (t, C-39), 30.0 (t, C-20), 30.3 (d, C-23), 30.5 (t, C-34), 37.2 (d, C-28), 41.2 (t, C-5), 44.9 (q, C-38), 48.6 (d, C-4), 53.1 (d, C-27), 55.4 (t, C-37), 69.7 (d, C-33), 71.8 (d, C-41), 79.4 (s, C-40), 122.7 (d, C-18), 128.3 (d, C-13), 129.4 (d, C-7), 129.9 (d, C-12), 130.4 (d, C-8), 132.4 (d, C-14), 134.6 (d, C-3), 136.0, 137.6 (2s, C-9, C-11), 142.0 (s, C-6), 149.2 (s, C-17), 160.4 (s, C-16), 167.9 (s, C-1), 169.9 (s, C-19), 173.4, 174.2 (2s, C-26, C-32), 196.3 (s, C-10).

Not observable: C-2, C-22

optical rotation: $[\alpha]^{20}_{D} = +25°$ (c = 0.23, $CHCl_3$)

HRMS (CI)	calculated	found
$C_{46}H_{61}N_6O_5S$ $[M+H]^+$	809.4424	809.4385

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