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Synthesis and structure–activity relationship studies of novel tubulysin U analogues – effect on cytotoxicity of structural variations in the tubuvaline fragment†

Sreejith P. Shankar,[a] Monika Jagodzinska,[a,b] Luciana Malpezzi,[a] Paolo Lazzari,[c] Ilaria Manca,[d] Iain R. Greig,[e] Monica Sani*[c,f] and Matteo Zanda*[c,e,f]

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Tubulysins are cytotoxic natural products with promising anti-cancer properties, originally isolated from myxobacterial cultures. Structurally, tubulysins are tetrapeptides, incorporating three unusual (Mep, Tuv and Tup) and one proteinogenic amino acid (Ile). Here we describe the synthesis and structure–activity relationship studies of novel tubulysin U and V analogues, with variations in the central Tuv fragment, which is known to be of paramount importance for tubulysins' potency and hence cytotoxicity, but has seldom been modified in previous studies. Specifically, we replaced the natural iso-propyl and acetoxy functionalities with other structurally related groups. In general, the new analogues showed much lower potency relative to native tubulysin U. However, one of the synthetic analogues (**1f**) having a MOM function replacing the acetyl group exhibited a 22 nM IC_{50} on the HT-29 cell line which is comparable to the IC_{50} displayed by tubulysin U (3.8 nM). Furthermore, the synthetic methodology reported herein was found to be flexible enough to deliver different core-modified tubulysin analogues and hence may be regarded as a scalable and convenient strategy for the chemical generation of novel tubulysin analogues.

Introduction

Tubulysins **1** (Fig. 1) are a family of tetrapeptides produced in rather small quantities (<4 mg L^{-1} culture broth) by two different species of *Myxobacteria*.[1] First isolated from the culture supernatant of an *Archangium gephyra* strain, tubulysins were later found in the fermentation broth of *Angiococcus disciformis*, which preferentially produced tubulysin D. Tubulysins are extremely toxic to mammalian cells, including multidrug-resistant cell lines, with IC_{50} values between 0.01 and 10 nM.[2] The cytotoxic activity of the tubulysins stems from their ability to bind tubulin and disintegrate microtubules of dividing cells, thus inducing apoptosis and hence the name.[3]

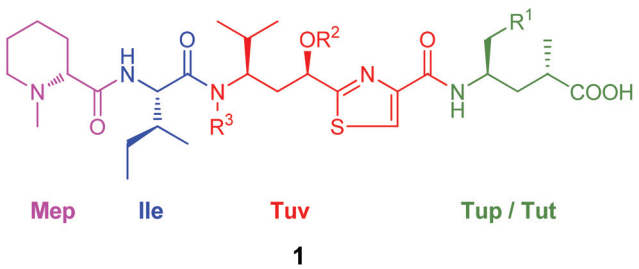

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Fig. 1 Structure of the tubulysins.

From a structural point of view, tubulysins are linear tetrapeptides incorporating L-isoleucine and three unnatural amino acids. At the N-terminus, all the family members have *N*-methyl pipecolic acid (Mep) and isoleucine (Ile, the only proteinogenic amino acid). The central position is occupied by the unusual amino acid tubuvaline (Tuv) containing a thiazole heterocycle. The tetrapeptide is completed at the C-terminus by either tubuphenylalanine (Tup, present in tubulysins D, E, F, H, U and V) or tubutyrosine (Tut, present in tubulysins A, B, C, G, I, Y and Z), which are γ-amino acid homologues of

[a]*Dipartimento di Chimica, Materiali e Ingegneria Chimica "Giulio Natta", Politecnico di Milano, Via Mancinelli 7, 20131 Milan, Italy*
[b]*Department of Pharmaceutical Chemistry, Jagiellonian University, Collegium Medicum, Medyczna 9 30-688 Krakow, Poland*
[c]*KemoTech s.r.l., Parco Scientifico della Sardegna, Edificio 3, Loc. Piscinamanna, 09010 Pula, CA, Italy*
[d]*C.N.R. Istituto di Farmacologia Traslazionale, UOS Cagliari, Edificio 5, Loc. Piscinamanna, 09010 Pula, CA, Italy*
[e]*Kosterlitz Centre for Therapeutics, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, Scotland, UK. http://www.abdn.ac.uk/kosterlitz/*
[f]*C.N.R. – Istituto di Chimica del Riconoscimento Molecolare, Sezione 'A. Quilico', Via Mancinelli 7, 20131 Milano, Italy*

†CCDC 853619 for **8a** and 853618 for **15b**. For crystallographic data in CIF or other electronic format see DOI: 10.1039/c3ob27111k

Table 1 The tubulysins family

Tubulysin	R^1	R^2	R^3
A	*p*-OH-Ph	Ac	$-CH_2OCO$-i-butyl
B	*p*-OH-Ph	Ac	$-CH_2OCO$-*n*-propyl
C	*p*-OH-Ph	Ac	$-CH_2OCOEt$
D	Ph	Ac	$-CH_2OCO$-i-butyl
E	Ph	Ac	$-CH_2OCO$-*n*-propyl
F	Ph	Ac	$-CH_2OCOEt$
G	*p*-OH-Ph	Ac	$-CH_2OCOCH{=}C(CH_3)_2$
H	Ph	Ac	$-CH_2OCOMe$
I	*p*-OH-Ph	Ac	$-CH_2OCOMe$
U	Ph	Ac	H
V	Ph	H	H
Y	*p*-OH-Ph	Ac	H
Z	*p*-OH-Ph	H	H

phenylalanine and tyrosine, respectively. Additionally, the N-terminal moiety of Tuv may be further functionalized with an unusual *N*,*O*-acetal substituent having different ester functionalities (tubulysins A–I) (Table 1). Tubulysins U–Z are devoid of such an *N*-Tuv substituent and as a consequence show lower cytotoxicity, albeit for tubulysin U this is still in the nanomolar range.

Although at first sight the tubulysins present a relatively simple linear tetrapeptide structure, the presence of six stereogenic centres and several chemically and configurationally-sensitive functionalities render their total synthesis a challenging endeavour. Due to their considerable interest and potential as powerful anticancer agents, several reports on the total synthesis of natural or modified tubulysins have been published.[4] Among them, one should mention the structurally simplified "Tubugis" compounds,[4m] "pre-tubulysins",[4i,n–p] oxo-tubulysins,[4c] *N*-methyl-tubulysins,[4d,f] and tubulysins U and V.[5,6] These modified tubulysin analogues are generally less potent than the more synthetically challenging natural tubulysins A and D, although they mostly display sub-nanomolar cytotoxicity on several different cancer cell lines. Remarkably, only a few papers describing scalable syntheses of the tubulysins have been published.[5] Last but not least, little information is available on the structural modification of the central Tuv fragment, which would be essential for drawing a reliable picture of the structure–activity relationship (SAR) features of the tubulysins, with the view of developing more synthetically accessible and chemically/metabolically stable tubulysin analogues for pre-clinical studies.

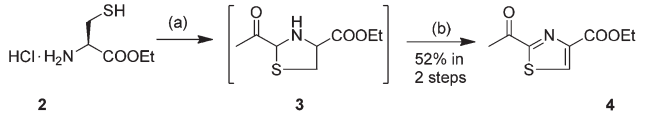
We have very recently reported the synthesis and cytotoxicity evaluation of an oxazole analogue of tubulysin U that showed slightly improved activity towards human promyelogenous leukaemic cells (HL-60),[4l] and this prompted us to evaluate the significance of the Tuv moiety in deciding the cytotoxicity of tubulysins. In this article we describe the total synthesis of tubulysin U and V analogues bearing structural modifications in the Tuv fragment, with the aim of carrying out an SAR study designed to shed light on the role and importance of the Tuv amino acid on the cytotoxic activity of the tubulysins.

Results and discussion

Back in 2007 we reported a scalable synthesis of tubulysins U and V.[6] With a reliable synthetic strategy in hand, we decided to explore whether the same could be extended to analogues of tubulysin U, preferably incorporating modified Tuv fragments. We decided to explore two different structural variations, namely (1) the replacement of the iso-propyl group by other alkyl and aryl groups and (2) the replacement of the *O*-acetyl moiety with alkoxy or benzoyl groups. This endeavour was undertaken in order to determine the importance and role of the central part of the tubulysins on their biological activity, with the view of using this knowledge in future studies on the key binding interactions with tubulin. So far the only available information on the role of the Tuv fragment was published by Wipf and co-workers,[7] who demonstrated that the natural (*R*)-configuration of the *O*-acetate is preferred and it influences tubulin assembly. Subsequently, Shibue *et al.* demonstrated that tubulysin D stereoisomers having different configurations of the Tuv stereogenic centres are equivalent to the natural isomer in terms of inhibition of tubulin polymerisation, but are considerably less cytotoxic, generally displaying an IC_{50} at least three orders of magnitude higher.[8] To the best of our knowledge, nothing has been published about the replacement of the Tuv iso-propyl group. However, a recent accurate NMR structural analysis of the tubulin-bound conformation of tubulysins suggested that the hydrophobic Tuv core of the tubulysins, which includes the iso-propyl group, plays an extremely important role in the binding process.[9]

Satisfactorily, we were able to extend our existing synthetic strategy,[6] with only slight modifications, to the generation of Tuv-analogues. Thus, the synthesis started from the condensation of L-cysteine hydrochloride ethyl ester **2** with methyl glyoxal in the presence of a base affording the thiazoline **3**, which was immediately oxidised using MnO_2 to the corresponding 2-acetyl thiazole ethyl ester **4** in 52% overall yield (Scheme 1).

Meanwhile, a one-pot reaction involving Boc-carbamate, benzene sulfinic acid sodium salt and the corresponding aldehyde in THF–H_2O or MeOH–H_2O afforded the α-amino sulfones **5a–d** (Scheme 2). Enolisation of the thiazole **4** using NaH followed by the addition of amino sulfones **5a–d** gave the β-amino ketones **6a–d** in satisfactory yields as racemic mixtures. In order to obtain stereochemically pure Tuv precursors, we performed an oxazoborolidine (CBS) mediated reduction[10] of **6a–d** to **7a–d** using the (*S*)-CBS catalyst in the presence of a $BH_3{\cdot}Me_2S$ complex at 0 °C. The required alcohols (*R*,*R*)-**7a–d**,



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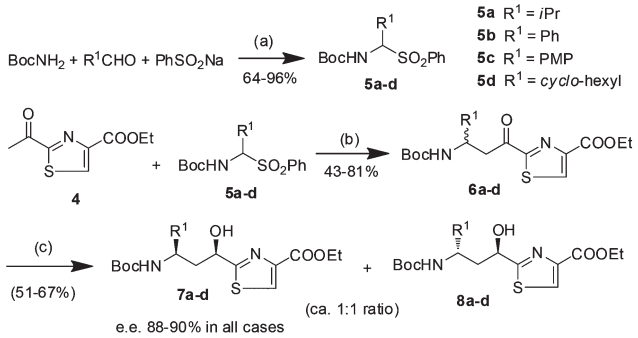
Scheme 1 Synthesis of 2-acetyl-thiazole ethyl ester **4**. Reagents and conditions: (a) methyl glyoxal solution, $NaHCO_3$, EtOH–H_2O (1 : 1), overnight; (b) MnO_2, MeCN, 65 °C, overnight.



Scheme 2 Synthesis of stereopure (*R*,*R*)-Tuv precursor. Reagents and conditions: (a) formic acid, THF–H_2O or MeOH–H_2O, 24 h; (b) NaH, THF, 2–3 h; (c) (*S*)-(−)-2-methyl-CBS-oxazaborolidine, BH_3·Me_2S, THF, 0 °C, 2–3 h.

which could be obtained in pure form by flash chromatography (FC), were produced along with the diastereoisomers **8a–d** in a *ca.* 1 : 1 ratio (Scheme 2), as determined by NMR analysis of the crude reaction mixtures.

Diastereomers **8**, with the exception of **8a**, could not be obtained in pure form by FC because they were always recovered as mixtures either with **7** or with unidentified by-products, which were initially not observed in the reduction of **6a**. Diastereomers **7a–d** were obtained in 88–90% ee, as determined by chiral HPLC analysis, whereas among diastereomers **8**, only **8a** could be submitted to chiral HPLC analysis, showing 96% ee.

The relative configuration of the two stereocenters in **8a** was confirmed by X-ray diffraction analysis (Fig. 2) of a suitable racemic crystal and the absolute configuration was assessed by chemical correlation with the enantiopure *O*-Ac-Cbz-Tuv-OEt described by Wipf *et al.*[11]

The next step consisted of the *O*-alkylation of the free hydroxy group in **7a**, so as to obtain the *O*-alkylated *N*,*C*-protected tubuvalines **7e,f**. This was achieved by reaction of methyl iodide or MOM-Cl with the corresponding potassium or sodium bases, respectively (Scheme 3).

The synthesis of the Tup unit is shown in Scheme 4. The key reaction for assembling the Tup fragment was a Wittig reaction between *N*-Boc protected phenylalanine aldehyde **9**[5] and the ylide **13**, which was chosen because we knew that *N*-Boc(−)-menthyl esters **15a** and **15b** could eventually be separated by FC. Starting from (−)-menthol, acylation with

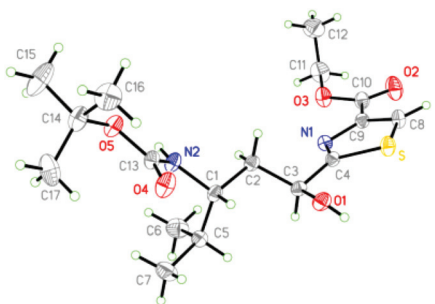
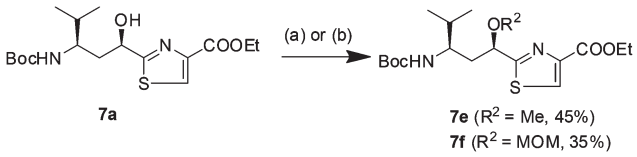
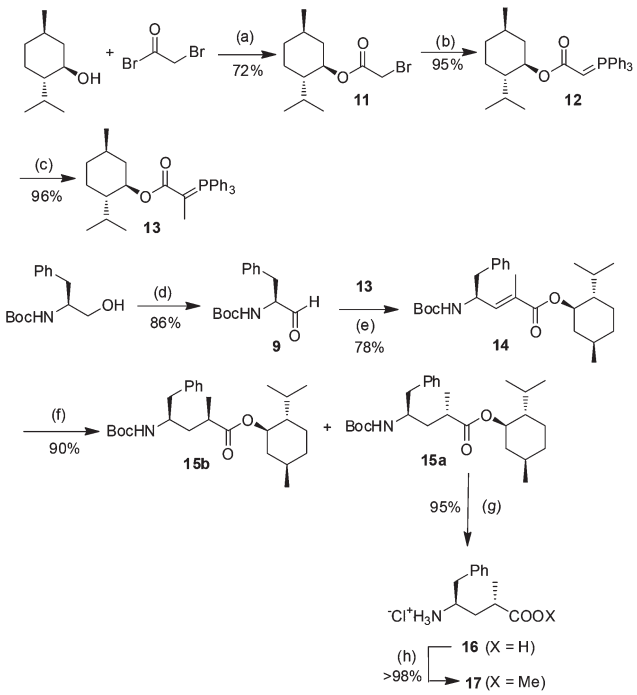


Fig. 2 X-ray structure of racemic **8a**.



Scheme 3 Synthesis of *O*-alkylated Tuv precursors. Reagents and conditions: (a) MeI, *t*BuOK, THF, −78 °C; (b) MOMCl, NaH, THF, 0 °C.



Scheme 4 Synthesis of stereopure Tup-OMe. Reagents and conditions: (a) Et_3N, dry THF, 0 °C to rt, 2 h; (b) PPh_3, THF, 2 h, 0.38 N NaOH, toluene, 3 h; (c) MeI, CH_2Cl_2, 0 °C to rt, overnight; (d) Dess–Martin periodinane, CH_2Cl_2, 6 h; (e) CH_2Cl_2, 0 °C to rt, 8 h; (f) H_2, Pd/C, EtOAc, overnight; (g) 6 N HCl, 130 °C, 1.5 h; (h) 2,2-dimethoxypropane, conc. HCl, MeOH, 60 °C, overnight.

bromoacetyl bromide followed by reaction with triphenylphosphine gave rise to the ylide **12**, which was reacted with methyl iodide to yield **13**, which was subjected to Wittig olefination with the aldehyde **9**. The latter was obtained by Dess–Martin oxidation of Boc-phenylalaninol **9**, affording the α,β unsaturated compound **14** in good yield. Hydrogenolysis of **14** with Pd/C provided a 2 : 1 diastereomeric mixture of Tup-derivatives **15**, separable by FC. One-pot acid hydrolysis of all the protecting groups gave stereochemically pure H-Tup-OH·HCl **16**, which was then esterified to yield H-Tup-OMe·HCl **17**, ready for subsequent couplings.

The relative stereochemistry of the two isomers was assigned based on X-ray analysis of the undesired diastereoisomer **15b** (Fig. 3).

Final assembly of tubulysins **1a–f** was achieved by a standard stepwise peptide synthesis protocol, as shown in Scheme 5. Boc deprotection of **7a–f** with 20% TFA in CH_2Cl_2 and subsequent coupling of the resulting amines **18a–f** with Boc-Ile-OH afforded the corresponding dipeptides **19a–f** in

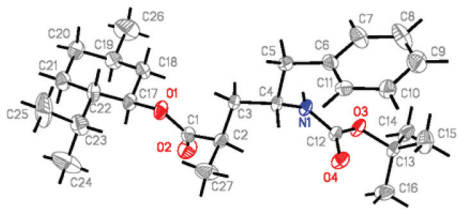
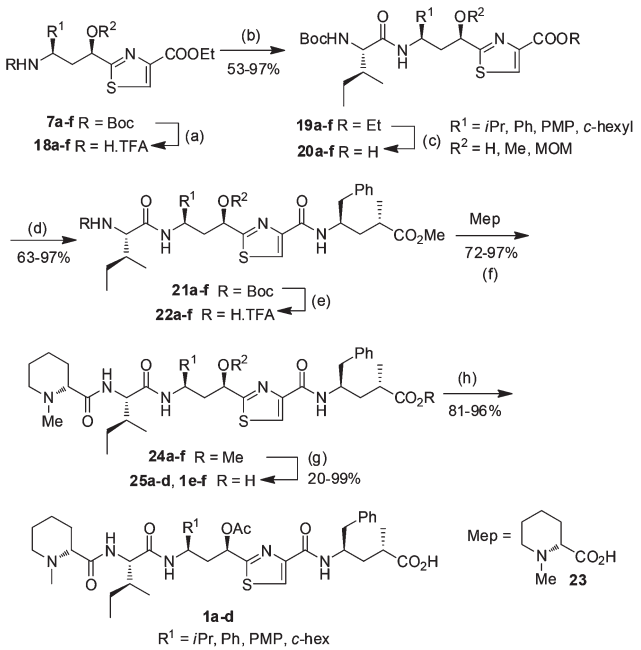


Fig. 3 X-ray structure of the *N*-boc-(–)-menthyl ester **15b**.



Scheme 5 Final assembling of fragments. Reagents and conditions: (a) TFA–CH_2Cl_2 (1 : 4), 0 °C to rt, 1 h; (b) HOBt, EDC·HCl, then Boc-Ile-OH, DIPEA, CH_2Cl_2, 0 °C to rt, 3 h; (c) LiOH·H_2O, THF–H_2O (4 : 1), 0 °C to rt, 5 h; (d) HOAt, HATU, then H-Tup-OMe (**17**), Et_3N, CH_2Cl_2, 0 °C to rt, 3 h; (e) TFA–CH_2Cl_2 (1 : 4), 0 °C to rt, 1 h; (f) HOAt, HATU, then Mep-OH (**23**), Et_3N, 0 °C to rt, 3 h; (g) 1 N LiOH, THF, 0 °C to rt, 2–3 days; (h) Ac_2O, pyridine, overnight.

satisfactory yields. Hydrolytic cleavage of ethyl ester under mild alkaline conditions delivered the acids **20a–f**, which, on subsequent coupling with H-Tup-OMe, **17**, afforded the tripeptides **21a–f**. Coupling between *N*-methyl pipecolic acid **23** and the tripeptides **22a–f**, obtained by treatment of **21a–f** with TFA, proceeded smoothly in the presence of HATU, HOAt and Et_3N, affording the tetrapeptides **24a–f** in good yields and without any detectable loss of stereochemical purity. Saponification of the methyl ester function using 1 M aqueous LiOH in THF gave the final carboxylic acid tubulysin U analogues **1e,f** and **25a–d** in good yields. The latter compounds were eventually acetylated by treatment with acetic anhydride in pyridine, affording the corresponding tubulysin U derivatives **1a–d** in stereopure form.

Eventually we accomplished the synthesis of the *O*-benzoyl derivative of tubulysin U, which turned out to be more challenging than expected. In fact, attempts to achieve direct benzoylation of the Tup hydroxy group were unsuccessful, possibly because of steric reasons. Furthermore the benzoate moiety proved to be labile even to the mild basic reaction conditions used for hydrolysing the C-terminal ethyl ester. Thus, we decided to modify the synthetic sequence starting from the Tuv stereoisomer *ent*-**8a**, as portrayed in Scheme 6. The racemic β-amino ketone **6a** was subjected to reduction using (*R*)-CBS catalyst, yielding a 1 : 1 diastereomeric mixture of alcohols *ent*-**7,8a**. Saponification of *ent*-**8a** with LiOH to **26** and subsequent coupling with Tup-OMe **17** afforded the corresponding dipeptide **27** in good yield. Next, a Mitsunobu reaction using PPh_3, benzoic acid and a 40% toluene solution of DEAD afforded the *O*-benzoyl derivative **28**, having the desired stereochemistry, resulting from inversion of configuration of the starting (*S*)-Tuv stereocenter. TFA mediated Boc deprotection of **28** to **29**, and subsequent coupling with Boc-Ile-OH gave the tripeptide **30**. After further N-deprotection, **31** was coupled with *N*-methyl pipecolic acid (**23**) affording the tetrapeptide **32**. Eventually, selective cleavage of the C-terminal methyl ester was accomplished using Me_3SnOH which was previously shown to be effective for a highly selective hydrolysis of tubulysin methyl esters.[8] Thus treatment of **32** with Me_3SnOH at 70 °C for 32 h delivered **1g**, albeit in modest yields, due to the formation of several unidentified by-products.

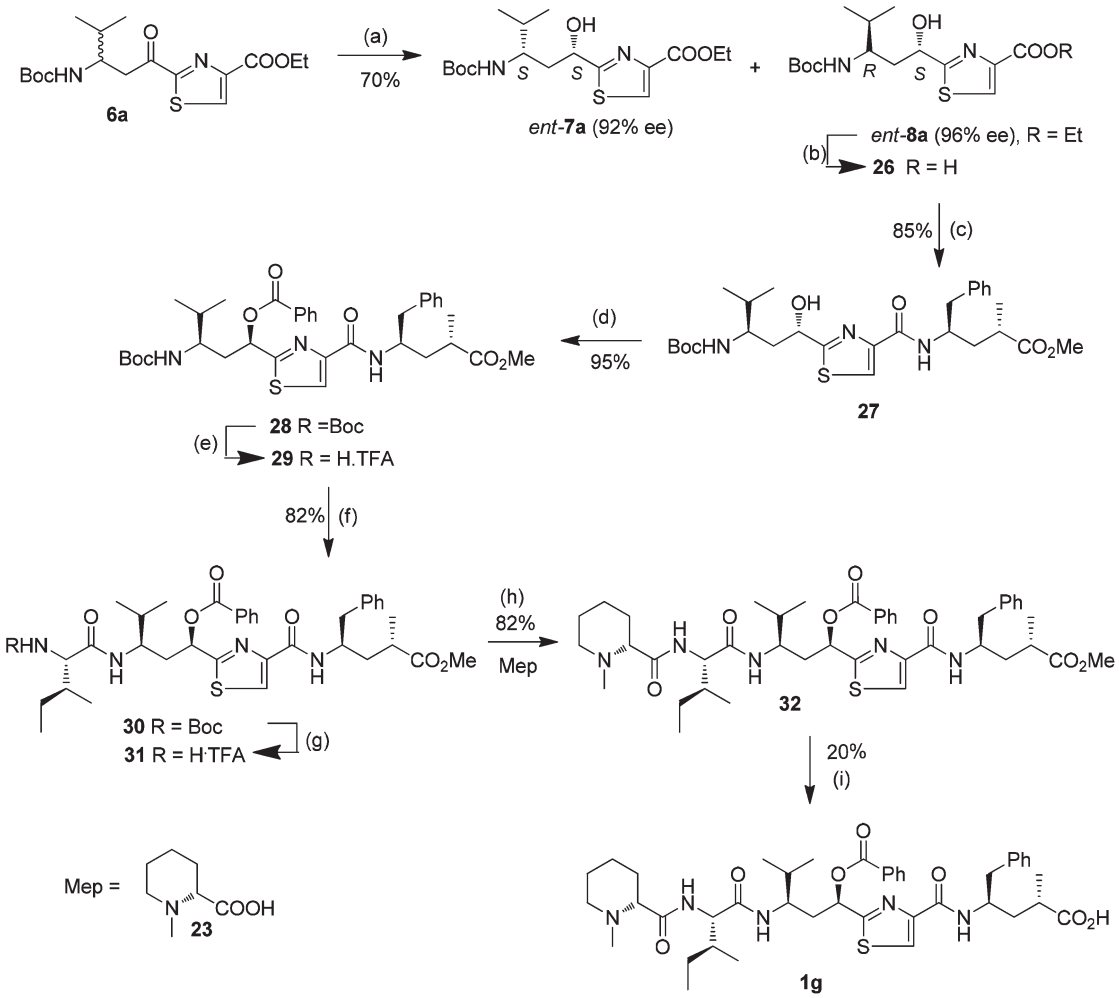
The tubulysin analogues **1a–f** were tested for their antitumour activity on the human colon cancer cell line HT-29 and the results are summarised in Table 2.

These data show that replacement of the acetyl group Tuv fragment of tubulysin U by a methyl group (compound **1e**) caused a two-fold loss of potency, in line with the previously observed decreased cytotoxicity of *O*-deacetylated tubulysin V,[5] whereas the methoxymethyl group produced a minimal drop of cytotoxicity and the resulting compound **1f** showed remarkable potency. These findings are very important because the acetyl group of tubulysins is hydrolytically- and metabolically-labile, and its replacement with a functionality less susceptible to hydrolysis could produce more stable and easier-to-handle tubulysin derivatives. Finally, replacement of the acetyl with the benzoyl group in compound **1g** also produced a drop of potency.

The inactivity of analogues **1b–d** demonstrates the importance of the iso-propyl group on the Tuv fragment – replacing the iPr group with other alkyl and aryl groups causes a dramatic drop of cytotoxicity. These finding are in line with the recently published SAR data analysis based on the tubulin-bound structure of the tubulysins determined by NMR structural analysis. Indeed, the Tuv iso-propyl belongs to the "hydrophobic core" of the tubulysins, that extends from the Ile side chain to the thiazole ring, which was correctly deemed to be essential by Carlomagno *et al.*[9]

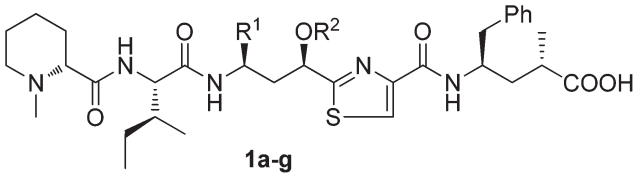
Conclusions

We developed a scalable and efficient total synthesis of tubulysins U and V,[6] which is flexible enough to be used for the synthesis of analogues incorporating different tubuvaline



Scheme 6 Synthesis of *O*-benzoyl tubulysin U analogue **1g**. Reagents and conditions: (a) (*R*)-(+)-2-methyl-CBS-oxazaborolidine, $BH_3{\cdot}Me_2S$, THF, 0 °C, 2–3 h; (b) $LiOH{\cdot}H_2O$, $THF–H_2O$ (4 : 1), 0 °C to rt; (c) HOAt, HATU, then H-Tup-OMe **17**, Et_3N, CH_2Cl_2, 0 °C to rt; (d) PPh_3, PhCOOH, DEAD, benzene; (e) $TFA–CH_2Cl_2$ (1 : 4), 0 °C to rt; (f) HOBt, EDC·HCl, then Boc-Ile, *sym*-collidine, CH_2Cl_2, 0 °C to rt; (g) $TFA–CH_2Cl_2$ (1 : 4), 0 °C to rt, 1 h; (h) HOAt, HATU, then Mep **23**, Et_3N, 0 °C to rt, 3 h; (i) Me_3SnOH, DCE, 70 °C, 32 h.

Table 2 Biological tests of the tubulysin analogues



Tubulysin analogue	IC_{50} (nM)
1a R^1 = iPr, R^2 = Ac	3.8
1b R^1 = Ph, R^2 = Ac	>1000
1c R^1 = PMP, R^2 = Ac	>1000
1d R^1 = *c*-hex, R^2 = Ac	>1000
1e R^1 = iPr, R^2 = Me	240
1f R^1 = iPr, R^2 = MOM	22
1g R^1 = iPr, R^2 = COPh	>1000

fragments in quantities and purities sufficient to allow *in vitro* biological screenings for cytotoxicity. An SAR study on a small set of novel tubulysin U analogues was performed. One of the synthesised analogues **1f**, containing an *O*-MOM replacement of the natural *O*-acetyl group on the central Tuv fragment, essentially retained the activity of the parent analogue **1a**. All the other replacements rendered the molecule biologically inactive or remarkably less active than the natural analogue, demonstrating the importance of the Tuv iso-propyl side-chain for the biological activity of the tubulysins.

Experimental section

General methods

Commercially-available reagent-grade solvents were employed without purification. All reactions where an organic solvent was employed were performed under a nitrogen atmosphere, after flame-drying of the glass apparatus. Melting points (m.p.) are uncorrected and were obtained on a capillary apparatus. TLCs were run on silica gel 60 F_{254} Merck. Flash

Chromatography (FC) purifications were performed with silica gel 60 (60–200 μm, Merck). ^{1}H-, ^{13}C-, and ^{19}F-NMR spectra were run at 250, 400 or 500 MHz. Chemical shifts are expressed in ppm (δ), using tetramethylsilane (TMS) as the internal standard for ^{1}H and ^{13}C nuclei (δ_H and δ_C = 0.00), while C_6F_6 was used as the external standard (δ_F −162.90) for ^{19}F.

Ethyl 2-acetylthiazole-4-carboxylate, 4. To a solution of cysteine hydrochloride ethyl ester **2** (15 g, 80.79 mmol) in a 1 : 1 EtOH–H_2O mixture (1.5 L) $NaHCO_3$ (6.786 g, 80.79 mmol) and pyruvic aldehyde (35% w in H_2O, 17.5 mL, 114 mmol) were added. The reaction mixture was stirred at rt for 18 h, then concentrated to half of its original volume (no heating). NaCl was added to saturate the aqueous phase. The aqueous layer was extracted with $CHCl_3$ (2 × 300 mL). The combined organic phase was dried over Na_2SO_4, concentrated *in vacuo* and the crude was used in the next step without any further purification.

To a solution of compound **3** (16.31 g, 80.74 mmol) in MeCN (500 mL) MnO_2 (140 g, 1.615 mol) was added. The reaction mixture was heated at 65 °C overnight, then filtered over a celite pad and the residue washed with AcOEt (2 × 200 mL). The filtrate was concentrated *in vacuo*. The crude was purified by FC (1 : 3 AcOEt–hexane) to give the acyl thiazole **4** (8.321 g, 52% in two steps) as a yellow solid. R_f = 0.33 (1 : 3 AcOEt–hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.40 (s, 1H), 4.43 (q, J = 7.1 Hz, 3H), 2.75 (s, 3H), 1.41 (t, J = 7.1 Hz, 3H). LC/MS (ESI) m/z 199.8 $[M + H]^+$, 221.8 $[M + Na]^+$. For a complete set of spectroscopic data, see ref. 12.

General procedure for the synthesis of the *tert*-butyl (phenylsulfonyl)methyl carbamates 5a–d. To a solution of *tert*-butyl carbamate (1 equiv.) in a H_2O–MeOH 1 : 2 mixture or H_2O–THF 1 : 2 were added the aldehyde (2 equiv.), benzene sulfinic acid sodium salt (2 equiv.) and formic acid (2 equiv.). The reaction mixture was stirred at rt for 24 h and cooled in an ice-bath. The white precipitate was filtered off, washed with water and hexane and dried to give the corresponding *tert*-butyl(phenylsulfonyl)methylcarbamate as a white solid and was used in the next step without further purification or characterisation.

General procedure for the preparation of β-amino carbonyl compounds, 6a–d. To a suspension of NaH (60% dispersion in mineral oil, 995 mg, 24.87 mmol) in dry THF (60 mL) acyl thiazole **4** (3 g, 15.07 mmol) was added. After stirring for 15 min a solution of sulfone **5a** (3.067 g, 9.8 mmol) in dry THF (60 mL) was added over a period of 30 min. The reaction was stirred for 2 h, quenched with an NH_4Cl saturated aqueous solution and extracted with AcOEt (2 × 50 mL). The collected organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by FC.

Ethyl 2-(3-(*tert*-butoxycarbonylamino)-4-methylpentanoyl)-thiazole-4-carboxylate, 6a. (White solid, 52% yield); R_f = 0.55 (4 : 6 AcOEt–hexane); FT-IR (film) ν_{max}: 3364.6, 2976.4, 1734.9, 1709.5, 1668.3, 1505.7, 1367.7 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.37 (s, 1H), 4.77 (br s, 1H), 4.39 (q, J = 6.9 Hz, 2H), 4.03–3.91 (m, 1H), 3.38–3.17 (m, 2H), 1.37 (t, J = 6.9 Hz, 3H), 1.32 (s, 9H), 0.92 (d, J = 6.6 Hz, 6H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 192.2, 167.3, 160.6, 155.4, 148.6, 133.0, 121.4, 61.6, 53.1, 41.4, 32.2, 28.2, 19.1, 14.1; LC/MS (ESI) m/z 393.4 $[M + Na]^+$.

Ethyl 2-(3-(*tert*-butoxycarbonylamino)-3-phenylpropanoyl)-thiazole-4-carboxylate, 6b. (Pale yellow solid, 43% yield); R_f = 0.36 (3 : 7 AcOEt–hexane); FT-IR (film) ν_{max}: 3363.4, 3110.1, 2979.4, 1706.3, 1495.9, 1366.7, 1218.6 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.47 (s, 1H), 7.69–6.95 (m, 5H), 5.46–5.33 (m, 2H), 4.51 (q, J = 7.2 Hz, 2H), 3.90–3.74 (m, 2H), 1.49 (t, J = 7.2 Hz, 3H), 1.46 (s, 9H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 191.6, 167.4, 161.1, 155.4, 149.1, 133.7, 129.0, 129.0, 127.9, 126.8, 126.4, 122.7, 62.2, 45.3, 30.0, 28.7, 14.7; LC/MS (ESI) m/z 427.1 $[M + Na]^+$.

Ethyl-2-(3-(*tert*-butoxycarbonylamino)-3-(4-methoxyphenyl)-propanoyl)thiazole-4-carboxylate, 6c. (White solid, 52% yield); R_f = 0.35 (35 : 65 AcOEt–hexane); FT-IR (film) ν_{max}: 3445.8, 3020.2, 1706.3, 1215.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.40 (s, 1H), 7.28 (d, J = 8.7 Hz, 2H), 6.83 (d, J = 8.7 Hz, 2H), 5.33–5.20 (m, 2H), 4.44 (q, J = 7.1 Hz, 2H), 3.82–3.62 (m, 2H), 3.76 (s, 3H), 1.42 (t, J = 7.1 Hz, 3H), 1.38 (s, 9H). ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 191.8, 167.5, 161.1, 159.3, 157.2, 155.4, 149.0, 133.8, 128.0, 114.4, 80.0, 62.3, 55.7, 51.0, 45.2, 28.7, 14.7; LC/MS (ESI) m/z 457.0 $[M + Na]^+$.

Ethyl 2-(3-(*tert*-butoxycarbonylamino)-3-cyclohexylpropanoyl)-thiazole-4-carboxylate, 6d. (White solid, 62% yield); R_f = 0.41 (3 : 7 AcOEt–hexane); FT-IR (film) ν_{max}: 3364.6, 2976.4, 1734.7, 1709.2, 1505.6, 1368.2 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.37 (s, 1H), 4.79 (d, J = 8.9 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 4.00–3.92 (m, 1H), 3.38–3.22 (m, 2H), 1.80–1.48 (m, 6H), 1.37 (t, J = 7.1 Hz, 3H), 1.32 (s, 9H), 1.22–0.96 (m, 5H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 192.8, 167.8, 161.1, 155.8, 149.0, 133.5, 79.4, 62.0, 52.7, 42.4, 41.7, 30.2, 29.2, 28.6, 26.6, 26.4, 26.4, 14.6; LC/MS (ESI) m/z 433.1 $[M + Na]^+$.

General procedure for the stereoselective reduction of 6a–d. To a solution of (*S*)-(−)-2-methyl-CBS-oxazaborolidine (152 mg, 0.55 mmol) in dry THF (20 mL) cooled at 0 °C, $BH_3{\cdot}Me_2S$ (10 M solution in THF, 499 μL, 4.99 mmol) was added. The solution was stirred for 10 min at 0 °C and a solution of **6a** (1.846 g, 4.99 mmol) in dry THF (10 mL) was added. The reaction was warmed to rt and stirred for 3 h. The reaction was quenched with MeOH (2 mL), the solvent removed *in vacuo* and the crude purified by FC.

Ethyl 2-((1*R*,3*R*)-3-(*tert*-butoxycarbonylamino)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate, 7a. (White solid, 39% yield) R_f = 0.51 (4 : 6 AcOEt–hexane); $[\alpha]_D^{23}$ = +4.5 (c = 1.74, $CHCl_3$); FT-IR (film) ν_{max}: 3375.1, 2965.9, 1734.3, 1660.4, 1505.6, 1391.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.08 (s, 1H), 5.14 (br s, 1H), 4.98 (br d, J = 10.7 Hz, 1H), 4.58 (d, J = 9.4 Hz, 1H), 4.36 (q, J = 7.3 Hz, 2H), 3.75–3.64 (m, 1H), 2.04 (dt, J = 12.1, 2.2 Hz, 1H,), 1.81–1.63 (m, 2H), 1.40 (s, 9H), 1.37 (t, J = 7.3 Hz, 3H), 0.93 (d, J = 7.0 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 176.6, 161.5, 157.9, 146.8, 127.1, 80.2, 69.1, 61.2, 52.3, 41.8, 32.2, 28.3, 19.3, 18.3, 14.3; LC/MS (ESI) m/z 395.1 $[M + Na]^+$.

Ethyl 2-((1*R*,3*R*)-3-(*tert*-butoxycarbonylamino)-1-hydroxy-3-phenylpropyl)thiazole-4-carboxylate, 7b. (Pale yellow solid, 36% yield); R_f = 0.26 (35 : 55 AcOEt–hexane); $[\alpha]_D^{23}$ = +72.38 (*c* = 0.6, $CHCl_3$); FT-IR (film) ν_{max}: 3349.9, 3119.3, 2979.1, 2929.5, 1716.6, 1497.1, 1391.8 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.09 (s, 1H), 7.34–7.16 (m, 5H), 5.25 (br s, 1H), 5.17 (d, *J* = 10.9 Hz, 1H), 5.08–4.99 (m, 2H), 4.39 (q, *J* = 6.9 Hz, 2H), 2.60–2.46 (m, 1H), 2.15–1.94 (m, 2H), 1.43 (s, 9H), 1.38 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 176.5, 161.9, 157.6, 147.3, 141.3, 129.3, 128.2, 127.6, 127.0, 81.0, 69.4, 61.7, 51.9, 45.8, 28.7, 14.7; LC/MS (ESI) *m*/*z* 407.0 $[M + H]^+$, 429.1 $[M + Na]^+$.

Ethyl 2-((1*R*,3*R*)-3-(*tert*-butoxycarbonylamino)-1-hydroxy-3-(4-methoxyphenyl)propyl)thiazole-4-carboxylate, 7c. (Pale yellow solid, 39% yield); R_f = 0.29 (4 : 6 AcOEt–hexane); $[\alpha]_D^{23}$ = +55.69 (*c* = 0.96, $CHCl_3$); FT-IR (film) ν_{max}: 3349.8, 3020.3, 1716.4, 1497.2 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.07 (s, 1H), 7.21 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 5.31 (br s, 1H), 5.21–5.01 (m, 2H), 4.97–4.92 (m, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 3.74 (s, 3H), 2.53–2.47 (m, 1H), 2.03–1.97 (m, 1H), 1.40 (s, 9H), 1.35 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 176.7, 161.9, 159.4, 157.5, 147.3, 133.4, 128.1, 127.6, 114.6, 80.8, 69.4, 61.7, 55.6, 51.3, 45.7, 28.7, 14.7; LC/MS (ESI) *m*/*z* 459.0 $[M + Na]^+$.

Ethyl 2-((1*R*,3*R*)-3-(*tert*-butoxycarbonylamino)-1-hydroxy-3-cyclohexylpropyl)thiazole-4-carboxylate, 7d. (White solid, 40% yield); R_f = 0.34 (45 : 55 AcOEt–hexane); $[\alpha]_D^{23}$ = +46.29 (*c* = 0.7, $CHCl_3$); FT-IR (film) ν_{max}: 3375.1, 2965.9, 1734.3, 1505.6, 1391.0 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.06 (s, 1H), 5.10 (br s, 1H), 4.96–490 (m, 1H), 4.70–4.56 (m, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 3.67–3.60 (m, 1H), 2.36–2.33 (m, 1H), 1.96–1.88 (m, 1H), 1.74–1.63 (m, 5H), 1.46 (br s, 1H), 1.37 (s, 9H), 1.35 (t, *J* = 7.1 Hz, 3H), 1.30–0.80 (m, 5H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 177.8, 161.9, 157.1, 146.9, 127.7, 80.3, 71.6, 61.6, 53.6, 43.1, 41.6, 30.0, 28.7, 28.4, 26.7, 26.5, 26.5, 14.7; LC/MS (ESI) *m*/*z* 413.1 $[M + H]^+$, 435.1 $[M + Na]^+$.

Ethyl 2-((1*R*,3*S*)-3-(*tert*-butoxycarbonylamino)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate, 8a. R_f = 0.27 (4 : 6 AcOEt–hexane); $[\alpha]_D^{23}$ = +57.7 (*c* = 1.2, $CHCl_3$); FT-IR (film) ν_{max}: 3370.7, 2966.5, 1737.8, 1717.3, 1648.9, 1506.4, 1368.3 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.07 (s, 1H), 5.18–5.04 (m, 1H), 4.82 (br s, 1H), 4.56 (br d, *J* = 8.6 Hz, 1H), 4.38 (q, *J* = 6.9 Hz, 2H), 3.68–3.56 (m, 1H), 2.39–2.26 (m, 1H), 1.98–1.77 (m, 2H), 1.39 (s, 9H), 1.37 (t, *J* = 6.9 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 177.4, 161.4, 156.5, 146.7, 127.0, 79.6, 70.8, 61.1, 53.4, 40.7, 32.3, 28.2, 1.9, 17.3, 14.2; LC/MS (ESI) *m*/*z* 395.1 $[M + Na]^+$.

Ethyl 2-((1*R*,3*S*)-3-(*tert*-butoxycarbonylamino)-1-methoxy-4-methylpentyl)thiazole-4-carboxylate, 7e. To a 1 M solution of *t*-BuOK (0.54 mL, 0.54 mmol) in dry THF (5 mL), cooled at −78 °C, a solution of **7a** (100 mg, 0.28 mmol) in dry THF (3 mL) was added. After 15 min, MeI (83 μL, 1.34 mmol) was added and the resulting mixture was stirred at −78 °C for 1 h. H_2O (6 mL) was added and the aqueous layer was extracted with EtOAc (3 × 10 mL). The solvent was removed *in vacuo* and the crude was purified by FC (2 : 8 AcOEt–hexane) affording **7e** (48 mg, 45%) as a yellowish foam. R_f = 0.54 (4 : 6 AcOEt–hexane); $[\alpha]_D^{23}$ = +13.6 (*c* = 0.95, $CHCl_3$); FT-IR (film) ν_{max}: 3350.7, 2945.5, 1682.9, 1650.2, 1530.2, 1239.8, 1170.1 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.13 (s, 1H), 4.60 (br d, *J* = 9.0 Hz, 1H), 4.42 (q, *J* = 7.1 Hz, 2H), 3.87 (m, 1H), 3.45 (s, 3H), 1.84 (dd, *J* = 14.3, 2.7 Hz, 1H), 1.78 (dd, *J* = 6.8, 2.9 Hz, 1H), 1.76–1.65 (m, 2H), 1.45 (s, 9H), 1.39 (t, *J* = 7.07 Hz, 3H), 0.90 (d, *J* = 6.75 Hz, 3H), 0.86 (d, *J* = 7.07 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 175.6, 161.3, 156.2, 147.6, 127.9, 79.2, 61.8, 59.2, 52.1, 41.7, 33.2, 28.8, 19.4; LC/MS (ESI) *m*/*z* 409.1 $[M + Na]^+$.

Ethyl 2-((1*R*,3*S*)-3-(*tert*-butoxycarbonylamino)-1-methoxymethyl-4-methylpentyl)thiazole-4-carboxylate, 7f. To a solution of alcohol **7a** (1.1 g, 2.96 mmol) in dry THF (50 mL) cooled at 0 °C, NaH (60% dispersion in mineral oil, 355 mg, 8.87 mmol) was added. The resulting suspension was stirred at 0 °C for 15 min, then neat MOMCl (1.12 mL, 14.8 mmol) was added. After stirring at rt for 3 h, the reaction was quenched by adding a 1 N HCl aqueous solution (20 mL). The layers were separated and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by FC to give **7f** (2 : 8 AcOEt–hexane) (428 mg, 35%) as a white foam. R_f = 0.43 (3 : 7 AcOEt–hexane); $[\alpha]_D^{23}$ = +12.6 (*c* = 0.75, $CHCl_3$); FT-IR (film) ν_{max}: 3342.6, 2932.5, 2879.9, 1680.8, 1650.2, 1528.2, 1235.8, 1171.1 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.97 (s, 1H), 4.84 (br d, *J* = 9.0 Hz, 1H), 4.57 (d, *J* = 6.7 Hz, 1H), 4.55 (d, *J* = 6.7 Hz, 1H), 4.48 (br d, *J* = 10.6 Hz, 1H), 4.22 (q, *J* = 7.06 Hz, 2H), 3.63 (m, 1H), 3.20 (s, 3H), 1.74 (m, 1H), 1.64 (m, 1H), 1.56 (m, 1H), 1.26 (s, 9H), 1.20 (t, *J* = 7.1 Hz, 3H), 0.72 (d, *J* = 6.7 Hz, 3H), 0.70 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 175.8, 161.5, 155.9, 147.2, 127.7, 97.6, 61.5, 56.9, 51.9, 40.9, 33.2, 28.5, 19.0, 17.9, 14.6; LC/MS (ESI) *m*/*z* 417.1 $[M + H]^+$, 439.1 $[M + Na]^+$.

(1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl 2-bromoacetate, 11. To a solution of menthol (5 g, 32 mmol) in dry THF (30 mL) Et_3N (4.8 mL, 35 mmol) was added. After cooling at 0 °C, bromoacetyl bromide (3 mL, 35 mmol) was added dropwise. The temperature was allowed to warm to rt and the reaction mixture was stirred for 2 h. After cooling at 0 °C, the reaction was quenched with a 1 N HCl aqueous solution (5 mL) and AcOEt was added (30 mL). The layers were separated and the organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo.* The crude was purified by FC (3 : 97 AcOEt–hexane) to give **11** (6.4 g, 72%) as a colorless oil. R_f = 0.5 (2 : 98 AcOEt–hexane); $[\alpha]_D^{23}$ = −64.1 (*c* = 1.68, $CHCl_3$); spectral data matches with those reported in the literature.[13]

(1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl 2-(triphenylphosphoranylidene) acetate, 12. To a solution of **11** (6.4 g, 23 mmol) in dry THF (30 mL), under a nitrogen atmosphere, PPh_3 (6 g, 23 mmol) was added. After refluxing for 2.5 h, the reaction mixture was concentrated *in vacuo*. The resulting solid was washed with a 7 : 3 mixture of hexane–Et_2O and filtered to give 11 g of phosphonium salt as a white solid, which was used in the next step without further purification. To a suspension of phosphonium salt (11 g, 23 mmol) in toluene (150 mL)

a 0.38 N NaOH aqueous solution (25 mL) was added dropwise over a period of 5 min. The reaction mixture was stirred for 3 h and the layers were separated. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give **12** (11 g, quantitative yield) as a white foam. R_f = 0.87 (3 : 7 MeOH–$CHCl_3$); $[\alpha]_D^{23}$ = −7.8 (*c* = 0.9, $CHCl_3$); FT-IR (film) ν_{max}: 3156.2, 3066.4, 1777.5, 1717.5, 1611.9, 1497.5 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.67–7.62 (m, 6H), 7.53–7.50 (m, 3H), 7.44–7.41 (m, 7H), 4.57–4.55 (m, 1H), 2.84–2.81 (m, 1H), 1.94–1.90 (m, 1H), 1.57–1.54 (m, 1H), 1.43–1.39 (m, 2H), 0.98–0.88 (m, 2H), 0.81–0.65 (m, 10H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 133.4, 133.3, 132.1, 129.0, 128.9, 71.2, 48.1, 42.3, 34.9, 31.7, 31.1, 29.8, 23.6, 22.5, 16.5; LC/MS (ESI) *m*/*z* 481.2 $[M + Na]^+$.

(1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl 2-(triphenylphosphoranylidene)propanoate, 13. To a suspension of phosphorane **12** (11 g, 23 mmol) in toluene (150 mL) a 0.38 N NaOH aqueous solution (25 mL) was added dropwise over a period of 5 min. The reaction mixture was stirred for 3 h and the layers were separated. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give 11 g of pure phosphonium salt as a white foam. To a solution of phosphonium salt (11 g, 23 mmol) in DCM (60 mL), cooled at 0 °C, MeI (2.1 mL, 34 mmol) was added dropwise. After stirring for 10 min the temperature was allowed to warm to rt. The reaction mixture was stirred overnight and the solvent was evaporated. The crude was dissolved in toluene (100 mL) and a 0.38 N NaOH aqueous solution (25 mL) was added. The mixture was stirred for 2 h and the layers were separated. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give pure **13** (10.4 g, 96%) as a yellow foam. R_f = 0.6 (2 : 8 MeOH–$CHCl_3$); $[\alpha]_D^{23}$ = −43.2 (*c* = 1.2, $CHCl_3$); FT-IR (film) ν_{max}: 3158.1, 3060.4, 1777.5, 1718.3, 1611.9, 1495.4 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.69–7.64 (m, 5H), 7.56–7.52 (m, 4H), 7.47–7.44 (m, 7H), 4.53–4.50 (m, 1H), 2.09–2.02 (m, 1H), 1.92–1.86 (m, 1H), 1.72–1.65 (m, 1H), 1.59 (s, 3H), 1.50–1.41 (m, 2H), 1.35–1.25 (m, 2H), 1.13–0.98 (m, 1H), 0.93–0.89 (m, 4H), 0.79–0.74 (m, 3H), 0.69–0.62 (m, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 133.8, 133.6, 132.1, 131.8, 128.6, 128.1, 46.1, 34.2, 31.2, 29.1, 26.2, 22.0, 21.1, 15.7; LC/MS (ESI) *m*/*z* 495.1 $[M + Na]^+$.

(1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl 4-(*tert*-butoxycarbonylamino)-2-methyl-5-phenylpent-2-enoate, 14. To a solution of **13** (4.53 g, 9.6 mmol) in DCM (80 mL), cooled at 0 °C, aldehyde **9**[5] (1.6 g, 6.4 mmol) was added. After stirring for 15 min at 0 °C, the temperature was allowed to warm to rt and the reaction mixture was stirred for 8 h. The reaction was quenched with a 1 N $NaHSO_4$ aqueous solution (50 mL) and extracted with DCM (2 × 50 mL). The organic layer was washed with brine (1 × 50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified by FC (2 : 8 AcOEt–hexane) to give **14** (2.3 g, 78%) as a white foam; R_f = 0.5 (3 : 7 AcOEt–hexane); $[\alpha]_D^{23}$ = −8.52 (*c* = 0.7, $CHCl_3$); FT-IR (film) ν_{max}: 3096.4, 2641.9, 1974.6, 1881.2, 1711.8, 1620.6, 1459.8 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.25–7.18 (m, 3H), 7.13–7.11 (m, 3H), 6.48–6.45 (m, 1H), 4.72–4.65 (m, 3H), 2.91 (dd, *J* = 13.3, 4.0 Hz, 1H), 2.76 (dd, *J* = 13.3, 6.7 Hz, 1H), 1.99–1.95 (m, 1H), 1.87–1.80 (m, 2H), 1.68 (s, 3H), 1.39 (s, 9H), 1.25–1.23 (m, 1H), 1.10–0.92 (m, 2H), 0.90–0.87 (m, 10H), 0.75 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 177.2, 155.2, 139.1, 134.3, 132.5, 129.6, 129.2, 128.9, 126.7, 66.8, 54.5, 47.5, 41.6, 41.4, 34.7, 31.6, 25.8, 23.3, 22.4, 21.4, 16.2; LC/MS (ESI) *m*/*z* 466.1 $[M + Na]^+$, 442.2 $[M + H]^+$.

(2*S*,4*R*)-((1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl) 4-(*tert*-butoxycarbonylamino)-2-methyl-5-phenylpentanoate, 15a–b. To a solution of **14** (2.1 g, 4.85 mmol) in EtOAc (20 mL), a catalytic amount of Pd/C was added. The reaction mixture was stirred under a hydrogen atmosphere overnight and then filtered through celite. The filtrate was concentrated under reduced pressure and the two diastereomers were separated by FC ($iPrO_2$–hexane 3 : 7) to give **15a** (950 mg) and **15b** (910 mg, 87% overall yield) as white solids; **15a**: R_f = 0.62 (1 : 4 AcOEt–hexane); $[\alpha]_D^{23}$ = −17.1 (*c* = 1.4, $CHCl_3$); m.p. = 87–88 °C; FT-IR (film) ν_{max} = 2958, 2931, 2871, 1704, 1498, 1454, 1390, 1365, 1253, 1173 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29–7.26 (m, 2H), 7.22–7.16 (m, 3H), 4.66 (dt, *J* = 10.8, 4.3 Hz, 1H), 4.34 (br s, 1H), 3.92–3.80 (br m, 1H), 2.80–2.70 (m, 2H), 2.62–2.50 (m, 1H), 2.02–1.96 (m, 1H), 1.91–1.81 (m, 2H), 1.71–1.64 (m, 2H), 1.54–1.35 (m, 4H), 1.39 (s, 9H), 1.15 (d, *J* = 7.0 Hz, 3H), 1.10–1.00 (m, 1H), 0.92–0.80 (m, 1H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.75 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 175.7, 155.1, 137.9, 129.5, 128.3, 126.3, 74.1, 49.9, 47.1, 41.2, 40.8, 37.8, 36.8, 34.3, 31.4, 29.7, 28.4, 26.3, 23.4, 22.0, 20.8, 17.7, 16.0; LC(MS (ESI) *m*/*z* 446.3 $[M + H]^+$, 468.4 $[M + Na]^+$. **15b**: R_f = 0.7 (1 : 4 AcOEt–hexane); $[\alpha]_D^{23}$ = −31.6 (*c* = 0.7, $CHCl_3$); FT-IR (film) ν_{max}: 2957.9, 2929.2, 1870.8, 1715.6, 1496.8 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.30–7.26 (m, 2H), 7.22–7.15 (m, 3H), 4.64 (dt, *J* = 10.8, 4.3 Hz, 1H), 4.34 (br s, 1H), 3.95–3.80 (br m, 1H), 2.83–2.73 (m, 2H), 2.50–2.41 (m, 1H), 1.98–1.92 (m, 1H), 1.86–1.60 (m, 4H), 1.54–1.31 (m, 4H), 1.39 (s, 9H), 1.13 (d, *J* = 7.0 Hz, 3H), 1.10–1.00 (m, 1H), 0.93–0.80 (m, 1H), 0.89 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.74 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 176.0, 155.3, 137.8, 129.4, 128.2, 126.2, 74.0, 49.7, 46.9, 42.0, 40.7, 37.2, 36.8, 34.1, 31.3, 29.6, 28.2, 26.1, 23.3, 21.9, 20.8, 16.7, 16.1; LC/MS (ESI) *m*/*z* 446.3 $[M + H]^+$, 468.3 $[M + Na]^+$.

(2*S*,4*R*)-Methyl 4-amino-2-methyl-5-phenylpentanoate hydrochloride, 17. A suspension of *N*-Boc menthyl ester **15a** (950 mg, 2.13 mmol) in 6 N HCl (10 mL) was refluxed at 145 °C for 3–5 h and then cooled to rt. AcOEt (10 mL) was added and the phases were separated. The aqueous layer was concentrated under reduced pressure to obtain Tup·HCl **16** (422 mg, 95%) as a white solid and was used in the next step without further purification. To a suspension of **16** (398 mg, 1.64 mmol) in MeOH (15 mL), 2,2-dimethoxypropane (405 μL, 3.28 mmol) followed by conc. HCl (4.8 μl, 0.015 mmol) were added. The reaction mixture was heated to 50 °C and stirred overnight. The solvent was removed *in vacuo* to give pure Tup-OMe **17** (383 mg, 91%) as a white foam; R_f = 0.3 (1 : 9 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ = +8.6 (*c* 1.0, MeOH); ^1H NMR (400 MHz, CD_3OD) δ: 7.46–7.15 (m, 5H), 3.63 (s, 3H), 3.59–3.47 (m, 1H),

3.04 (dd, J = 19.9, 6.2 Hz, 1H), 2.91 (dd, J = 13.7, 7.7 Hz, 1H), 2.79–2.65 (m, 1H), 2.08–1.95 (m, 1H), 1.16 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100.5 MHz, CD_3OD) δ: 178.1, 137.8, 131.3, 130.9, 129.3, 53.3, 41.1, 37.9, 37.7, 18.7; LC/MS (ESI) m/z 221.9 $[M + H]^+$.

General procedure for coupling Tuv 7a–f with Boc-Ile. A solution of **7a** (150 mg, 0.40 mmol) in a 20% mixture of TFA in DCM (5 mL) was stirred for 1 h. The solvent was removed under reduced pressure to give **18a** as the TFA salt and was used in the next step without further purification. To a solution of Boc-isoleucine (93 mg, 0.40 mmol) in DCM (5 mL), HOBt (60 mg, 0.44 mmol), EDC·HCl (84 mg, 0.44 mmol), DIPEA (153 μL, 0.88 mmol) and Tuv-TFA salt **18a** (160 mg, 0.40 mmol) were added. After stirring for 3 h water (10 mL) was added and the layers separated. The organic phase was washed with a 1 N HCl aqueous solution (10 mL), a saturated $NaHCO_3$ aqueous solution (10 mL) and brine (10 mL). After drying over anhydrous Na_2SO_4 and concentration *in vacuo* the crude dipeptide was purified by FC.

Ethyl 2-((1*R*,3*R*)-3-((2*S*,3*S*)-2-(*tert*-butoxycarbonylamino)-3-methylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate, 19a. (White foam, 80% yield); R_f = 0.47 (4 : 6 AcOEt–hexane); $[\alpha]_D^{23}$ = −3.1 (c = 1.1, $CHCl_3$); FT-IR (film) ν_{max}: 3419.0, 1651.6, 1497.9, 1216.0, 1165.2, 1099.8 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.08 (s, 1H), 6.35 (d, J = 8.1 Hz, 1H), 5.01 (d, J = 7.8 Hz, 1H), 4.86 (d, J = 10.0 Hz, 1H), 4.37 (q, J = 7.1 Hz, 2H), 4.15–3.88 (m, 2H), 2.17 (t, J = 10.1 Hz, 1H), 1.83–1.81 (m, 1H), 1.80–1.73 (m, 2H), 1.51 (s br, 1H), 1.41 (s, 9H), 1.35 (t, J = 7.1 Hz, 3H), 1.16–1.09 (m, 2H), 1.01 (d, J = 6.5 Hz, 3H), 0.97–0.83 (m, 9H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 176.8, 174.3, 161.9, 156.3, 147.4, 127.6, 80.0, 69.2, 61.7, 52.1, 41.2, 36.0, 32.4, 28.6, 25.0, 19.8, 18.6, 16.3, 14.7, 11.4; LC/MS (ESI) m/z 486.2 $[M + H]^+$, 508.0 $[M + Na]^+$.

Ethyl 2-((1*R*,3*R*)-3-((2*S*,3*S*)-2-(*tert*-butoxycarbonylamino)-3-methylpentanamido)-1-hydroxy-3-phenylpropyl)thiazole-4-carboxylate, 19b. (Pale yellow foam, 75% yield); R_f = 0.42 (1 : 1 AcOEt–hexane); $[\alpha]_D^{23}$ = +65.67 (c = 0.34, $CHCl_3$); IR (film) ν_{max}: 3419.0, 3020.1, 1651.5, 1497.9, 1369.0, 1216.0 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09 (s, 1H), 7.37–7.23 (m, 5H), 6.85 (d, J = 7.1 Hz, 1H), 5.37 (d, J = 3.5 Hz, 1H), 5.29 (ddd, J = 11.3, 8.3, 3.0 Hz, 1H), 5.05–5.01 (m, 1H), 4.92 (d, J = 8.4 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 4.01–3.95 (m, 1H), 2.66 (ddd, J = 14.1, 11.8, 2.6 Hz, 1H), 2.10 (ddd, J = 14.0, 11.0, 3.0 Hz, 1H), 1.97–1.91 (m, 1H), 1.49 (br s, 1H), 1.38 (t, J = 7.1 Hz, 3H), 1.37 (s, 9H), 1.19–1.05 (m, 1H), 0.95 (d, J = 6.8 Hz, 3H), 0.89 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 176.3, 173.5 (2C), 161.9, 147.4, 140.7, 129.3, 128.3, 127.7, 127.1, 69.2, 61.7, 60.1, 51.1, 44.3, 36.6, 30.0, 28.6, 25.1, 16.3, 14.7, 11.6; LC/MS (ESI) m/z 520.2 $[M + H]^+$, 542.2 $[M + Na]^+$.

Ethyl 2-((1*R*,3*R*)-3-((2*S*,3*S*)-2-(*tert*-butoxycarbonylamino)-3-methylpentanamido)-1-hydroxy-3-(4-methoxyphenyl)propyl)-thiazole-4-carboxylate, 19c. (Pale yellow foam, 63% yield); R_f = 0.22 (1 : 1 AcOEt–hexane); $[\alpha]_D^{23}$ = +78.95 (c = 0.57, $CHCl_3$); IR (film) ν_{max}: 3410.0, 3020.2, 1651.6, 1497.2 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09: (s, 1H), 7.23 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 8.7 Hz, 2H), 6.73 (d, J = 7.4 Hz, 1H), 5.41 (d, J = 4.3 Hz, 1H), 5.24 (ddd, J = 11.3, 8.3, 2.9 Hz, 1H), 5.05–4.98 (m, 1H), 4.91 (d, J = 8.1 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 3.99–3.92 (m, 1H), 3.77 (s, 3H), 2.65 (ddd, J = 14.1, 11.8, 2.6 Hz, 1H), 2.07 (ddd, J = 14.1, 11.1, 2.7 Hz, 1H), 1.97–1.90 (m, 1H), 1.48 (br s, 1H), 1.38 (s, 9H), 1.38 (t, J = 7.1 Hz, 3H), 1.18–1.10 (m, 1H), 0.94 (d, J = 6.8 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 176.4, 173.4, 161.9, 159.7, 147.4, 132.8, 128.3, 127.7, 122.7, 114.7, 69.2, 61.7, 60.0, 55.7, 50.5, 44.2, 36.7, 30.0, 28.6, 25.1, 16.3, 14.7, 11.6; LC/MS (ESI) m/z 572.2 $[M + Na]^+$.

Ethyl 2-((1*R*,3*R*)-3-((2*S*,3*S*)-2-(*tert*-butoxycarbonylamino)-3-methylpentanamido)-1-hydroxy-3-cyclohexylpropyl)thiazole-4-carboxylate, 19d. (Off-white foam, 97% yield); R_f = 0.52 (1 : 1 AcOEt–hexane); $[\alpha]_D^{23}$ = +30.59 (c = 1.79, $CHCl_3$); IR (film) ν_{max}: 3419.4, 3020.1, 1651.6, 1369.0, 1216.0, 1165.2, 1099.8 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.05 (s, 1H), 6.45 (d, J = 7.5 Hz, 1H), 5.23 (d, J = 8.2 Hz, 1H), 4.97 (d, J = 8.3 Hz, 1H), 4.37 (q, J = 7.1 Hz, 2H), 4.09–3.93 (m, 1H), 3.77–3.63 (m, 1H), 2.42 (dd, J = 14.6, 2.4 Hz, 1H), 1.90–1.38 (m, 9H), 1.36 (s, 9H), 1.35 (t, J = 7.1 Hz, 3H), 1.20–0.95 (m, 7H), 0.92 (d, J = 6.7 Hz, 3H), 0.86 (t, J = 7.9 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 178.3, 172.7, 161.9, 156.9, 147.4, 127.8, 80.8, 71.4, 61.6, 60.7, 53.6, 42.9, 41.2, 36.8, 30.1, 28.7, 28.6, 26.7, 26.5, 26.5, 19.6, 16.1, 14.7, 14.4, 11.4; LC/MS (ESI): m/z 526.2 $[M + H]^+$, 548.2 $[M + Na]^+$.

Ethyl 2-((1*R*,3*R*)-3-((2*S*,3*S*)-2-(*tert*-butoxycarbonylamino)-3-methylpentanamido)-1-methoxy-4-methylpentyl)thiazole-4-carboxylate, 19e. (White foam, 58% yield); R_f = 0.28 (1 : 3 AcOEt–hexane); $[\alpha]_D^{23}$ = +1.9 (c = 0.62, $CHCl_3$); FT-IR (film) ν_{max}: 3367.8, 2963.1, 1716.4, 1501.4, 1366.3, 1237.3, 1173.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09 (s, 1H), 6.25 (d, J = 9.3 Hz, 1H), 4.98 (br d, 1H), 4.53 (dd, J = 9.32, 3.54 Hz, 1H), 4.38 (q, J = 7.1 Hz, 2H), 4.12 (m, 1H), 3.85 (t, J = 7.8 Hz, 1H), 3.41 (s, 3H), 1.91 (dd, J = 14.4, 3.53 Hz, 1H), 1.85 (dd, J = 8.9, 3.6 Hz, 1H), 1.82–1.75 (m, 2H), 1.54 (m, 1H), 1.39 (s, 9H), 1.36 (t, J = 7.1 Hz, 3H), 1.13 (m, 1H), 0.94 (d, J = 6.75 Hz, 3H), 0.90–0.86 (m, 9H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 175.8, 171.9, 161.6, 156.2, 147.6, 127.9, 79.1, 61.7, 59.1, 51.2, 40.1, 36.8, 30.1, 32.5, 28.6, 25.1, 19.2, 18.4, 16.3, 14.7, 11.5; LC/MS (ESI) m/z 500.1 $[M + H]^+$, 522.1 $[M + Na]^+$.

Ethyl 2-((1*R*,3*R*)-3-((2*S*,3*S*)-2-(*tert*-butoxycarbonylamino)-3-methylpentanamido)-1-methoxymethyloxy-4-methylpentyl)-thiazole-4-carboxylate, 19f. (White foam, 58%); R_f = 0.20 (3 : 7 AcOEt–hexane), $[\alpha]_D^{23}$ = +3.1 (c = 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06 (s, 1H), 6.25 (br d, J = 8.1 Hz, 1H), 5.03 (d, J = 8.7 Hz, 1H), 4.87 (t, J = 6.4 Hz, 1H), 4.70 (d, J = 6.5 Hz, 1H), 4.66 (d, J = 6.5 Hz, 1H), 4.35 (q, J = 7.0 Hz, 2H), 4.06 (m, 1H), 3.81 (t, J = 8.0 Hz, 1H), 3.34 (s, 3H), 1.92 (t, J = 6.6 Hz, 2H), 1.87–1.75 (m 2H), 1.51 (m, 1H), 1.35 (s, 9H), 1.33 (t, J = 7.4 Hz, 3H), 1.10 (m, 1H), 0.90 (d, J = 6.7 Hz, 3H), 0.87–0.81 (m, 9H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 175.5, 172.1, 161.6, 156.2, 147.5, 127.7, 97.5, 75.2, 61.6, 60.1, 57.1, 51.1, 39.7, 32.6, 28.6, 25.1, 18.9, 18.2, 16.1, 14.6, 11.5; LC/MS (ESI) m/z 530.2 $[M + H]^+$, 552.2 $[M + Na]^+$.

General procedure for coupling Ile-Tuv-COOEt 19a–f with Tup-OMe 17. To a solution of dipeptide **19a** (210 mg, 0.45 mmol) in a 4 : 1 THF–H_2O mixture (5 mL), LiOH·H_2O

(28 mg, 0.67 mmol) was added. The reaction mixture was stirred for 5 h. H_2O (5 mL) and AcOEt (10 mL) were added and the layers were separated. The aqueous phase was acidified to pH 1–2 with a 1 N HCl aqueous solution and was extracted with AcOEt (2 × 10 mL). The combined organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give the corresponding acid **20a**, which was used in the next step without further purification. To a solution of **20a** (180 mg, 0.40 mmol) in DCM (5 mL), HOAt (60 mg, 0.44 mmol), HATU (167 mg, 0.44 mmol), Et_3N (123 μL, 0.88 mmol) and Tup-OMe **17** (103 mg, 0.40 mmol) were added. After stirring for 3 h water (10 mL) was added and the layers separated. The organic phase was washed with a 1 N HCl aqueous solution (10 mL), a saturated $NaHCO_3$ aqueous solution (10 mL) and brine (10 mL). After drying over anhydrous Na_2SO_4 and concentration *in vacuo* the crude tripeptide was purified by FC.

(2*S*,4*R*)-Methyl 4-(2-((6*S*,9*R*,11*R*)-6-((*S*)-*sec*-butyl)-9-isopropyl-2,2-dimethyl-4,7,13-trioxo-3,12-dioxa-5,8-diazatetradecan-11-yl)-thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 21a. (Pale yellow foam, 63% yield); R_f = 0.42 (2 : 3 AcOEt–hexane); $[\alpha]_D^{23}$ = +15.2 (*c* = 0.7, $CHCl_3$); FT-IR (film) ν_{max}: 3290.4, 3020.0, 2960.5, 1729.7, 1645.6, 1541.7 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.00 (s, 1H), 7.31–7.13 (m, 5H), 7.08 (br s, 1H), 6.36 (d, *J* = 8.9 Hz, 1H), 5.23 (br s, 1H), 4.88 (br d, *J* = 10.4 Hz, 1H), 4.60 (d, *J* = 9.2 Hz, 1H), 4.45–4.32 (m, 1H), 3.89–3.68 (m, 2H), 3.62 (s, 3H), 2.93 (dd, *J* = 10.3, 6.1 Hz, 1H), 2.85 (dd, *J* = 13.5, 6.1 Hz, 1H), 2.66–2.54 (m, 1H), 2.07–1.89 (m, 3H), 1.88–1.71 (m, 3H), 1.67–1.48 (m, 2H), 1.43 (s, 9H), 1.15 (d, *J* = 7.1 Hz, 3H), 1.00–0.88 (m, 12H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 176.4, 175.5, 174.5, 160.7, 157.7, 149.6, 137.4, 129.5, 128.2, 126.3, 122.8, 80.2, 68.7, 52.3, 52.6, 48.2, 42.3, 41.4, 40.9, 37.7, 37.1, 36.4, 32.2, 28.2, 18.2, 17.6, 16.2, 14.5, 11.5; LC/MS (ESI) *m*/*z* 662.9 $[M + H]^+$, 684.8 $[M + Na]^+$.

(2*S*,4*R*)-Methyl 4-(2-((6*S*,9*R*,11*R*)-6-((*S*)-*sec*-butyl)-2,2-dimethyl-4,7,13-trioxo-9-phenyl-3,12-dioxa-5,8-diazatetradecan-11-yl)-thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 21b. (Pale yellow foam, 66% yield); R_f = 0.43 (65 : 35 AcOEt–hexane); $[\alpha]_D^{23}$ = + 35.9 (*c* = 1.18, $CHCl_3$); FT-IR (film) ν_{max}: 3313.0, 3028.5, 2966.1, 2931.8, 1656.9, 1540.8, 1496.7, 1366.9, 1247.9 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.97 (s, 1H), 7.33–7.05 (m, 12H), 5.66–5.25 (m, 2H), 5.22–5.09 (m, 1H), 4.93 (d, *J* = 10.5 Hz, 1H), 4.51–4.34 (m, 1H), 4.07–3.94 (m, 1H), 3.49 (s, 3H), 2.96–2.77 (m, 2H), 2.55–2.30 (m, 2H), 2.16 (t, *J* = 12.2 Hz, 1H), 1.96–1.85 (m, 3H), 1.63–1.42 (m, 2H), 1.38 (s, 9H), 1.13 (d, *J* = 6.4 Hz, 3H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 177.5, 175.6, 173.2, 161.3, 150.0, 141.0, 137.8, 129.9, 129.3, 128.8, 128.2, 127.0, 126.9, 123.7, 122.7, 68.9, 51.9, 50.9, 48.5, 44.4, 42.2, 39.0, 37.9, 37.1, 30.0, 28.6, 25.2, 17.7, 17.3, 16.2, 11.6; LC/MS (ESI) *m*/*z* 717.2 $[M + Na]^+$.

(2*S*,4*R*)-Methyl 4-(2-((6*S*,9*R*,11*R*)-6-((*S*)-*sec*-butyl)-9-(4-methoxyphenyl)-2,2-dimethyl-4,7,13-trioxo-3,12-dioxa-5,8-diazatetradecan-11-yl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 21c. (Pale yellow foam, 97% yield); R_f = 0.41 (65 : 35 AcOEt–hexane); $[\alpha]_D^{23}$ = +67.33 (*c* = 0.9, $CHCl_3$); FT-IR (film) ν_{max}: 3349.4, 3064.1, 1729.1, 1652.0, 1084.1 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.33–7.15 (m, 7H), 7.10 (d, *J* = 7.1 Hz, 1H), 6.90 (d, *J* = 8.1 Hz, 2H), 6.80 (d, *J* = 7.4 Hz, 1H), 5.41–5.19 (m, 2H), 4.96–4.91 (m, 2H), 4.39 (d, *J* = 4.6 Hz, 1H), 3.98 (t, *J* = 7.1 Hz, 1H), 3.80 (s, 3H), 3.61 (s, 3H), 3.00–2.80 (m, 2H), 2.64–2.54 (m, 1H), 2.46 (t, *J* = 12.6 Hz, 1H), 2.14 (t, *J* = 12.2 Hz, 1H), 2.04–1.88 (m, 3H), 1.61–1.44 (m, 2H), 1.38 (s, 9H), 1.14 (d, *J* = 6.9 Hz, 3H), 0.96 (d, *J* = 6.6 Hz, 3H), 0.90 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 176.9, 175.1, 173.2, 161.1, 159.7, 156.6, 150.2, 137.9, 137.8, 132.0, 130.0, 128.7, 128.2, 126.8, 123.5, 114.8, 69.0, 55.7, 52.0, 50.3, 48.7, 44.5, 41.5, 38.2, 36.9, 36.6, 30.0, 28.6, 25.2, 18.1, 17.2, 16.3, 11.7; LC/MS (ESI) *m*/*z* 747.2 $[M + Na]^+$.

(2*S*,4*R*)-Methyl 4-(2-((6*S*,9*R*,11*R*)-((*S*)-*sec*-butyl)-9-cyclohexyl-2,2-dimethyl-4,7,13-trioxo-3,12-dioxa-5,8-diazatetradecan-11-yl)-thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 21d. (Off-white foam, 75% yield). R_f = 0.47 (65 : 35 AcOEt–hexane); $[\alpha]_D^{23}$ = +12.73 (*c* = 0.22, $CHCl_3$); FT-IR (film) ν_{max}: 3295.4, 3025.2, 2960.5, 1729.7, 1645.6, 1541.7 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.94 (s, 1H), 7.35–7.11 (m, 5H), 6.44 (br s, 1H), 5.41 (br s, 1H), 5.25 (d, *J* = 7.1 Hz, 1H), 4.74–4.84 (m, 1H), 4.42–4.31 (m, 1H), 4.16–4.05 (m, 1H), 3.82–3.58 (m, 2H), 3.61 (s, 3H), 2.98–2.86 (m, 2H), 2.66–2.54 (m, 1H), 2.26 (d, *J* = 14.5 Hz, 1H), 2.11–1.96 (m, 2H), 1.95–1.41 (m, 9H), 1.36 (s, 9H), 1.30–0.95 (m, 6H), 1.15 (d, *J* = 7.1 Hz, 3H), 0.97 (d, *J* = 6.6 Hz, 3H), 0.89 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 177.0, 176.6, 172.6, 161.3, 150.4, 138.2, 129.9, 128.7, 126.8, 123.2, 80.8, 71.0, 60.9, 53.2, 52.0, 48.9, 43.3, 41.5, 41.1, 38.4, 36.9, 30.1, 28.9, 28.6, 26.8, 26.6, 26.5, 25.5, 18.1, 16.2, 14.4, 11.4; LC/MS (ESI): *m*/*z* 723.2 $[M + Na]^+$.

(2*S*,4*R*)-Methyl 4-(2-((3*R*,5*R*,8*S*)-8-((*S*)-*sec*-butyl)-5-isopropyl-12,12-dimethyl-7,10-dioxo-2,11-dioxa-6,9-diazatridecan-3-yl)-thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 21e. (White foam, 78% yield); R_f = 0.35 (2 : 3 AcOEt–hexane); $[\alpha]_D^{23}$ = −6.8 (*c* = 0.9, $CHCl_3$); FT-IR (film) ν_{max}: 3318.6, 2964.7, 2933.4, 1651.8, 1539.8, 1495.4, 1249.4; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.01 (s, 1H), 7.26–7.18 (m, 5H), 7.08 (d, *J* = 9.6 Hz, 1H), 5.06 (bd, 1H), 4.44 (d, *J* = 7.7 Hz, 1H), 4.15 (m, 1H), 3.88 (t, *J* = 7.8 Hz, 1H), 3.52 (s, 3H), 3.42 (s, 3H), 2.95 (dd, *J* = 13.5, 5.8 Hz, 1H), 2.81 (dd, *J* = 13.5, 7.2 Hz, 1H), 2.5 (m, 1H), 2.01–1.86 (m, 3H), 1.81–1.75 (m, 2H), 1.67–1.60 (m, 1H), 1.57 (m, 1H), 1.41 (s, 9H), 1.14 (d, *J* = 6.74 Hz, 3H), 0.96 (d, *J* = 6.74 Hz, 3H), 0.90 (m, 12H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 177.4, 174.5, 171.8, 171.8, 161.0, 156.4, 150.4, 138.0, 129.8, 128.8, 126.8, 123.7, 78.9, 59.0, 51.8, 51.1, 48.7, 42.4, 40.7, 37.9, 37.1, 36.8, 30.1, 32.5, 28.8, 25.1, 19.5, 18.3, 17.2, 16.3, 11.6; LC/MS (ESI) *m*/*z* 698.2 $[M + Na]^+$, 714.2 $[M + K]^+$.

(2*S*,4*R*)-Methyl 4-(2-((5*R*,7*R*,10*S*)-10-((*S*)-*sec*-butyl)-7-isopropyl-14,14-dimethyl-9,12-dioxo-2,4,13-trioxa-8,11-diazapentadecan-5-yl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 21f. (White foam, 84%); R_f = 0.32 (3 : 7 AcOEt–hexane); $[\alpha]_D^{23}$ = −7.1 (*c* = 1.28, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.01 (s, 1H), 7.28–7.20 (m, 5H), 7.11 (d, *J* = 9.6 Hz, 1H), 6.07 (br s, 1H), 4.97 (m, 1H), 4.84 (dd, *J* = 9.9, 2.2 Hz, 1H), 4.76 (d, *J* = 6.7 Hz, 1H), 4.71 (d, *J* = 6.7 Hz, 1H), 4.45 (m, 1H), 4.14 (m, 1H), 3.86 (t, *J* = 8.0 Hz, 1H), 3.54 (s, 3H), 3.38 (s, 3H), 2.98 (dd,

J = 13.8, 5.6 Hz, 1H), 2.84 (dd, J = 13.5, 7.4 Hz, 1H), 2.54–2.48 (m, 1H), 2.04–1.82 (m, 6H), 1.69–1.57 (m, 2H), 1.43 (s, 9H), 1.16 (d, J = 6.7 Hz, 3H), 0.97 (d, J = 6.7 Hz, 3H), 0.93–0.82 (m, 9H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 177.3, 174.2, 171.8, 161.0, 156.4, 150.3, 138.1, 129.8, 128.8, 126.9, 123.6, 97.5, 74.7, 60.2, 57.0, 51.9, 51.1, 48.7, 42.4, 40.5, 37.9, 37.1, 32.6, 30.0, 25.2, 19.3, 18.2, 17.3, 16.2, 11.5; LC/MS (ESI) m/z 727.2 $[M + Na]^+$.

Ethyl 2-((1*S*,3*S*)-3-(*tert*-butoxycarbonylamino)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate, *ent*-7a. The reduction was carried out with (*R*)-CBS catalyst according to the same procedure as described for the reduction of **6a–d** to **7a–d** (white solid, 35% yield); R_f 0.50 (2 : 3 AcOEt–hexane); $[\alpha]_D^{23}$ = −5.0 (c = 1.70, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.08 (s, 1H), 5.14 (br s, 1H), 4.98 (br d, J = 10.7 Hz, 1H), 4.58 (d, J = 9.4 Hz, 1H), 4.36 (q, J = 7.3 Hz, 2H), 3.75–3.64 (m, 1H), 2.04 (dt, J = 12.1, 2.2 Hz, 1H), 1.81–1.63 (m, 2H), 1.40 (s, 9H), 1.37 (t, J = 7.3 Hz, 3H), 0.93 (d, J = 7.0 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 176.6, 161.5, 157.9, 146.8, 127.1, 80.2, 69.1, 61.2, 52.3, 41.8, 32.2, 28.3, 19.3, 18.3, 14.3; LC/MS (ESI): m/z 395.1 $[M + Na]^+$.

Ethyl 2-((1*S*,3*R*)-3-(*tert*-butoxycarbonylamino)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate, *ent*-8a. (White foam, 35% yield); R_f 0.24 (3 : 2 hexane–AcOEt); ee = 96% (determined by HPLC analysis with CHIRACEL OD stationary phase, *n*-Hex–i-PrOH 9 : 1, 1.0 mL min^{-1}); $[\alpha]_D^{23}$ = −64.3 (c = 0.10, $CHCl_3$); m.p. = 76–78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.07 (s, 1H), 5.18–5.04 (m, 1H), 4.82 (br s, 1H), 4.56 (br m, 1H), 4.38 (q, J = 6.9 Hz, 2H), 3.68–3.56 (m, 1H), 2.49–2.41 (m, 1H), 2.39–1.77 (m, 2H), 1.39 (s, 9H), 1.37 (t, J = 7.3 Hz, 3H), 0.94 (d, J = 7.0 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 177.4, 161.4, 156.5, 146.7, 127.0, 79.6, 70.8, 61.1, 53.4, 40.7, 32.3, 28.2, 19.2, 17.3, 14.2; LC/MS (ESI): m/z 395.1 $[M + Na]^+$.

4-{[2-(3-*tert*-Butoxycarbonylamino-1-hydroxy-4-methyl-pentyl)-thiazole-4-carbonyl]-amino}-2-methyl-5-phenylpentanoic acid methyl ester, 27. To a solution of *ent*-**8a** (212 mg, 0.57 mmol) in a 4 : 1 THF–H_2O mixture (5 mL), $LiOH{\cdot}H_2O$ (34 mg, 0.85 mmol) was added. The reaction mixture was stirred for 5 h. H_2O (5 mL) and AcOEt (10 mL) were added and the layers were separated. The aqueous phase was acidified to pH 1–2 with a 1 N HCl aqueous solution and was extracted with AcOEt (2 × 10 mL). The combined organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give the corresponding acid **26**, which was used in the next step without further purification. To a solution of **26** (138 mg, 0.40 mmol) in DCM (5 mL), HOAt (60 mg, 0.44 mmol), HATU (167 mg, 0.44 mmol), Et_3N (123 μL, 0.88 mmol) and Tup-OMe **17** (103 mg, 0.40 mmol) were added. After stirring for 3 h water (10 mL) was added and the layers separated. The organic phase was washed with a 1 N HCl aqueous solution (10 mL), a saturated $NaHCO_3$ aqueous solution (10 mL) and brine (10 mL). After drying over anhydrous Na_2SO_4 and concentration *in vacuo* the crude was purified by FC to give the dipeptide **27** (186 mg, 85%) as a white foam. R_f = 0.35 (1 : 1 AcOEt–hexane); $[\alpha]_D^{23}$ = −32.8 (c = 0.51, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.49 (br d, 1H), 7.24–7.20 (m, 5H), 5.02 (d, J = 5.4 Hz, 1H), 4.80 (br d, J = 10.1 Hz, 1H), 4.67 (d, J = 9.2 Hz, 1H), 4.38–4.30 (m, 1H), 3.77–3.71 (m, 1H), 3.58 (s, 3H), 2.97 (dd, J = 13.3, 4.9 Hz, 1H), 2.80 (dd, J = 13.3, 7.0 Hz, 1H), 2.66–2.54 (m, 1H), 2.15–2.11 (m, 1H), 2.00–1.88 (m, 2H), 1.80–1.74 (m, 1H), 1.66 (m, 1H), 1.39 (s, 9H), 1.13 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 7.9 Hz, 3H), 0.90 (d, J = 8.6 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 177.1, 176.2, 161.3, 156.9, 150.2, 138.2, 129.8, 128.8, 126.8, 123.2, 80.0, 70.9, 53.4, 52.0, 49.2, 41.7, 41.2, 37.7, 36.9, 33.2, 28.7, 19.5, 18.0; LC/MS (ESI) m/z 548.1 $[M + H]^+$, 570.2 $[M + Na]^+$.

(1*R*,3*R*)-3-(*tert*-Butoxycarbonylamino)-1-(4-((2*R*,4*S*)-5-methoxy-4-methyl-5-oxo-1-phenylpentan-2-ylcarbamoyl)thiazol-2-yl)-4-methylpentyl benzoate, 28. To a solution of **27** (120 mg, 0.21 mmol) in benzene (5 mL), PPh_3 (144 mg, 0.54 mmol) and benzoic acid (66 mg, 0.54 mmol) were added followed by DEAD (40% solution in toluene, 249 μL, 0.54 mmol). The reaction mixture was stirred at rt for 45 min and then a $NaHCO_3$ saturated aqueous solution was added (10 mL). The layers were separated and the organic layer was concentrated *in vacuo*. The crude was purified *via* FC (3 : 7 AcOEt–hexane) affording **28** (129 mg, 95%) as a white foam. R_f = 0.47 (2 : 3 AcOEt–hexane); $[\alpha]_D^{23}$ = −3.84 (c = 0.26, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.07 (d, J = 7.3 Hz, 2H), 8.01 (s, 1H), 7.58–7.45 (m, 3H), 7.23–7.18 (m, 5H), 6.50 (br s, 2H), 6.35 (br s, 1H), 4.54 (d, J = 9.2 Hz, 1H), 4.40–4.34 (m, 1H), 3.85–3.73 (m, 1H), 3.61 (s, 3H), 2.66–2.32 (m, 2H), 2.03–1.97 (m, 2H), 1.88–1.70 (m, 2H), 1.61 (m, 1H), 1.36 (s, 9H), 1.14 (d, J = 7.0 Hz, 3H), 0.95 (d, J = 6.6 Hz, 3H), 0.93 (d, J = 7.3 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 177.0, 176.1, 170.4, 160.8, 157.1, 155.7, 150.3, 138.0, 136.5, 133.8, 130.2, 129.8, 128.8, 126.8, 123.8, 80.9, 70.2, 52.9, 52.0, 49.1, 41.4, 41.2, 37.6, 36.9, 33.3, 28.2, 19.0, 18.1; LC/MS (ESI) m/z 674.1 $[M + Na]^+$.

(1*R*,3*R*)-3-((2*S*,3*S*)-2-(*tert*-Butoxycarbonylamino)-3-methylpentanamido)-1-(4-((2*R*,4*S*)-5-methoxy-4-methyl-5-oxo-1-phenylpentan-2-ylcarbamoyl)thiazol-2-yl)-4-methylpentyl benzoate, 30. A solution of **28** (120 mg, 0.18 mmol) in a 20% mixture of TFA in DCM (5 mL) was stirred for 1 h. The solvent was removed *in vacuo* to give **29**, which was used in the next step without further purification. To a solution of **29** (120 mg, 0.18 mmol) in DCM (5 mL), HOAt (27 mg, 0.20 mmol), HATU (70 mg, 0.20 mmol), Et_3N (54 μL, 0.40 mmol) and Boc-Ile (46 mg, 0.20 mmol) were added. After stirring for 3 h water (10 mL) was added and the layers separated. The organic phase was washed with a 1 N HCl aqueous solution (10 mL), a saturated $NaHCO_3$ aqueous solution (10 mL) and brine (10 mL). After drying over anhydrous Na_2SO_4 and concentration *in vacuo*, the crude was purified by FC to give the tripeptide **30** (white foam, 82%). R_f = 0.67 (1 : 1 AcOEt–hexane); $[\alpha]_D^{23}$ = −13.3 (c = 0.59, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 7.9 Hz, 2H), 8.01 (s, 1H), 7.57 (t, J = 7.4 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.30–7.16 (m, 6H), 6.90 (br s, 1H), 6.32 (d, J = 9.2 Hz, 1H), 6.20 (dd, J = 9.5, 3.5 Hz, 1H), 5.93 (br d, J = 10.4 Hz, 1H), 5.07 (d, J = 8.9 Hz, 1H), 4.15 (d, J = 9.2 Hz, 1H), 4.37 (m, 1H), 3.85–3.75 (m, 1H), 3.60 (s. 3H), 2.94 (dd, J = 13.6, 6.0 Hz, 1H), 2.85 (dd, J = 13.6, 7.0 Hz, 1H), 2.64–2.56 (m, 1H), 2.37–2.30 (m, 1H),

2.18–2.11 (m, 1H), 2.03–1.96 (m, 1H), 1.91–1.79 (m, 2H), 1.65–1.58 (m, 1H), 1.56–1.48 (m, 1H), 1.40 (s, 9H), 1.14 (d, J = 7.0 Hz, 3H), 0.95 (d, J = 5.7 Hz, 3H), 0.93 (d, J = 6.3 Hz, 3H), 0.89 (d, J = 6.9 Hz, 3H), 0.85 (d, J = 7.3 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 176.9, 172.2, 170.3, 165.8, 160.8, 156.5, 150.3, 138.1, 133.8, 130.2, 129.8, 129.7, 128.8, 126.8, 125.4, 123.9, 79.1, 70.8, 62.4, 52.0, 51.0, 49.0, 41.6, 40.9, 38.1, 36.8, 32.2, 30.0, 28.6, 25.2, 19.3, 18.1, 16.0, 14.7; EI MS m/z: 787.2 $[M + Na]^+$.

General procedure for coupling the tripeptide Ile-Tuv-Tup-OMe with Mep. A solution of tripeptide **21a** (150 mg, 0.27 mmol) in a 20% mixture of TFA in DCM (5 mL) was stirred for 1 h. The solvent was removed *in vacuo* to give **22a**, which was used in the next step without further purification. To a solution of **22a** (100 mg, 0.18 mmol) in DCM (5 mL), HOAt (27 mg, 0.2 mmol), HATU (76 mg, 0.2 mmol), Mep (*N*-methyl-(*R*)-pipecolic acid (28 mg, 0.18 mmol) and Et_3N (50 μL, 0.36 mmol) were added. The reaction mixture was stirred for 3 h and then washed with a 1 N HCl aqueous solution (10 mL), a saturated $NaHCO_3$ aqueous solution (10 mL) and brine (10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude tetrapeptide **35a–e** thus obtained was purified using silica gel chromatography.

(2*S*,4*R*)-Methyl 4-(2-((1*R*,3*R*)-1-hydroxy-4-methyl-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)-pentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 24a. (White foam, 60% yield) R_f = 0.39 (1 : 9 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ = +35.3 (c 0.6, $CDCl_3$); FT-IR (film) ν_{max}: 3290.4, 2960.5, 1729.7, 1645.6, 1541.7 cm^{-1}; ^{1}H NMR (400 MHz, [D_6]DMSO) δ: 8.08 (s, 1H), 7.75 (d, J = 8.9 Hz, 1H), 7.66 (d, J = 9.6 Hz, 1H), 7.51 (br d, J = 8.9 Hz, 1H), 7.30–7.10 (m, 5H), 6.15 (d, J = 5.6 Hz, 1H), 4.73–4.65 (m, 1H), 4.24–4.12 (m, 2H), 4.03–3.91 (m, 1H), 3.52 (s, 3H), 2.92–2.74 (m, 3H), 2.57–2.50 (m, 1H), 2.47–2.38 (m, 1H), 2.11 (s, 3H), 2.03–1.69 (m, 6H), 1.68–1.26 (m, 7H), 1.28–1.08 (m, 2H), 1.06 (d, J = 6.8 Hz, 3H), 0.93–0.76 (m, 12H); ^{13}C NMR (100.5 MHz, [D_6]DMSO) δ: 177.6, 175.4, 170.9, 159.8, 14.2, 138.1, 128.8, 127.8, 125.8, 122.8, 68.7, 67.5, 56.5, 50.9, 49.9, 47.8, 43.6, 40.3, 37.2, 35.9, 35.5, 31.4, 29.4, 24.5, 24.2, 22.6, 18.7, 17.7, 17.2, 15.2, 10.1; LC/MS (ESI) m/z 686.3 $[M + H]^+$.

(2*S*,4*R*)-Methyl 4-(2-((1*R*,3*R*)-1-hydroxy-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)-3-phenylpropyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 24b. (Pale yellow foam, 72% yield); R_f = 0.33 (5 : 95 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ = +30.4 (c = 0.5, $CHCl_3$); FT-IR (film) ν_{max}: 3304.5, 3064.0, 2925.5, 1729.2, 1651.5, 1540.3, 1216.0 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.00 (s, 1H), 7.42–7.18 (m, 10H), 7.08 (d, J = 9.7 Hz, 1H), 5.36–5.26 (m, 1H), 4.91 (d, J = 10.6 Hz, 1H), 4.54–4.25 (m, 2H), 3.51 (s, 3H), 2.99–2.80 (m, 3H), 2.55–2.44 (m, 2H), 2.37–1.84 (m, 5H), 2.03 (s, 3H), 1.82–1.16 (m, 8H), 1.16 (d, J = 6.8 Hz, 3H), 1.00 (d, J = 5.6 Hz, 3H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ: 175.4, 172.8, 161.2, 150.1, 140.9, 137.9, 131.2, 130.0, 129.3, 128.8, 128.3, 127.0, 126.9, 123.6, 122.7, 69.0, 55.7, 51.9, 51.2, 48.4, 44.7, 42.2, 37.9, 37.2, 32.3, 30.1, 29.7, 25.3, 23.0, 17.4, 16.6, 16.4, 14.9, 14.4, 11.3; LC/MS (ESI): m/z 720.2 $[M + H]^+$, 742.2 $[M + Na]^+$.

(2*S*,4*R*)-Methyl 4-(2-((1*R*,3*R*)-1-hydroxy-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)-3-(4-methoxyphenyl)propyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 24c. (Pale yellow foam, 75% yield); R_f = 0.40 (1 : 9 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ = +72.29 (c = 0.83, $CHCl_3$); FT-IR (film) ν_{max}: 3340.2, 3064.1, 1729.1, 1652.0, 1084.1 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ: 8.02 (s, 1H), 7.29–7.11 (m, 7H), 6.84 (d, J = 8.6 Hz, 2H), 5.28 (dd, J = 9.6, 3.8 Hz, 1H), 4.90 (dd, J = 9.5, 3.4 Hz, 1H), 4.36–4.31 (m, 1H), 4.28–4.22 (m, 1H), 3.74 (s, 3H), 3.56 (s, 3H), 2.96–2.83 (m, 2H), 2.65–2.39 (m, 3H), 2.21–2.12 (m, 1H), 2.10 (s, 3H), 2.05–1.80 (m, 3H), 1.73–1.42 (m, 7H), 1.32–1.16 (m, 3H), 1.12 (d, J = 7.1 Hz, 3H), 0.97 (d, J = 6.7 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100.5 MHz, CD_3OD) δ: 179.3, 178.8, 176.4, 173.6, 163.6, 161.0, 151.4, 139.9, 136.1, 131.1, 130.0, 129.4, 128.1, 125.3, 115.6, 71.2, 70.3, 59.8, 59.6, 57.2, 56.5, 52.9, 51.6, 50.8, 45.8, 45.4, 43.0, 39.6, 38.4, 38.1, 32.1, 26.7, 24.9, 18.8, 16.9, 11.7; LC/MS (ESI): m/z 750.3 $[M + H]^+$, 772.3 $[M + Na]^+$.

(2*S*,4*R*)-Methyl 4-(2-((1*R*,3*R*)-1-hydroxy-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)-3-cyclohexylpropyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 24d. (Pale yellow foam, 97% yield); R_f = 0.47 (1 : 9 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ = +19.23 (c = 0.52, $CHCl_3$); FT-IR (film) ν_{max}: 3291.6, 3021.1, 2960.5, 1729.7, 1645.6 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ: 7.89 (s, 1H), 7.33–7.07 (m, 5H), 4.85–4.81 (m, 1H), 4.34–4.26 (m, 1H), 4.24–4.14 (m, 1H), 4.16–4.10 (m, 1H), 3.61 (s, 3H), 3.01–2.80 (m, 3H), 2.71–2.59 (m, 1H), 2.55–2.43 (m, 1H), 2.14 (s, 3H), 2.07–1.83 (m, 6H), 1.81–1.50 (m, 11H), 1.48–0.71 (m, 8H), 1.17 (d, J = 7.1 Hz, 3H), 0.98 (dd, J = 6.6 Hz, 3H), 0.90 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100.5 MHz, CD_3OD) δ: 181.6, 180.7, 178.9, 175.5, 166.1, 165.9, 142.5, 133.5, 132.2, 130.4, 122.7, 73.6, 73.3, 62.5, 59.6, 55.6, 55.2, 53.4, 48.2, 47.0, 45.3, 44.0, 42.5, 40.9, 40.7, 34.8, 34.5, 34.0, 32.7, 30.6, 30.4, 29.2, 26.8, 25.0, 19.4, 17.3, 12.1; LC/MS (ESI) m/z 727.3 $[M + H]^+$, 749.3 $[M + Na]^+$.

(2*S*,4*R*)-Methyl 4-(2-((1*R*,3*R*)-1-methoxy-4-methyl-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)-pentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 24e. (White foam, 82%); R_f = 0.60 (8 : 92 MeOH–$CHCl_3$), $[\alpha]_D^{23}$ = −2.1 (c = 0.62, $CHCl_3$); FT-IR (film) ν_{max}: 3275.1, 2935.8, 1735.5, 1642.1, 1541.8, 1494.6 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.01 (s, 1H), 7.28–7.19 (m, 5H), 7.11 (d, J = 9.6 Hz, 1H), 7.04 (d, J = 8.7 Hz, 1H), 6.19 (d, J = 9.9 Hz, 1H), 4.47–4.39 (m, 2H), 4.20–4.13 (m, 2H), 3.53 (s, 3H), 3.43 (s, 3H), 2.97 (dd, J = 13.7, 5.6 Hz, 1H), 2.94–2.88 (m, 1H), 2.83 (dd, J = 13.8, 7.4 Hz, 1H), 2.56–2.51 (m, 2H), 2.22 (s, 3H), 2.10–2.04 (m, 2H), 1.99–1.87 (m, 2H), 1.80–1.49 (m, 6H), 1.43–1.33 (m, 1H), 1.22–1.17 (m, 1H), 1.15 (d, J = 7.1 Hz, 3H), 1.12 (d, J = 6.4 Hz, 3H), 1.00 (d, J = 6.7 Hz, 3H), 0.98–0.82 (m, 9H); 13C NMR (100.6 MHz, $CDCl_3$) δ: 177.0, 175.0, 174.3, 170.8, 160.7, 150.1, 137.7, 129.5, 128.4, 126.5, 123.3, 78.6, 69.6, 58.7, 57.8, 55.4, 51.5, 50.7, 48.3, 44.9, 42.0, 40.4, 37.5, 36.7, 35.3, 32.2, 30.8, 25.1, 24.9, 23.3, 19.0, 17.9, 16.9, 16.1, 10.8; LC/MS (ESI) m/z 700.3 $[M + H]^+$, 722.3 $[M + Na]^+$.

(2*S*,4*R*)-Methyl 4-(2-((1*R*,3*R*)-1-methoxymethyloxy-4-methyl-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)-pentanamido)pentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 24f. (White foam, 74%); R_f = 0.71 (8 : 92 MeOH–$CHCl_3$); $[\alpha]_D^{23}$ = −11.2 (*c* = 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.99 (s, 1H), 7.25–7.14 (m, 5H), 7.02 (d, *J* = 8.7 Hz, 1H), 6.20 (d, *J* = 9.6 Hz, 1H), 4.80 (dd, *J* = 9.9, 2.6 Hz, 1H), 4.74 (d, *J* = 6.7 Hz, 1H), 4.69 (d, *J* = 6.7 Hz, 1H), 4.43–4.36 (m, 1H), 4.16–4.10 (m, 2H), 3.61 (s, 3H), 3.37 (s, 3H), 2.95–2.80 (m, 3H), 2.63–2.57 (m, 1H), 2.49 (dd, *J* = 11.2, 3.2 Hz, 1H), 2.20 (s, 3H), 2.04–1.95 (m, 4H), 1.88–1.74 (m, 3H), 1.70–1.35 (m, 5H), 1.28–1.17 (m, 2H), 1.14 (d, *J* = 7.0 Hz, 3H,), 0.96 (d, *J* = 6.7 Hz, 3H), 0.92–0.84 (m, 9H); ^{13}C NMR (100.5 MHz, $CDCl_3$) *δ*: 176.8, 175.4, 174.1, 171.3, 160.9, 150.5, 138.1, 129.8, 128.4, 126.8, 123.5, 97.4, 74.6, 70.0, 58.2, 57.0, 55.7, 52.0, 51.0, 48.9, 45.3, 41.7, 40.5, 38.2, 36.8, 35.6, 32.6, 31.1, 25.5, 23.6, 19.2, 18.1, 16.3, 14.4, 11.1; LC/MS (ESI) *m*/*z* 752.2 $[M + Na]^+$.

(2*S*,4*R*)-Methyl-4-(2-((1*R*,3*R*)-1-benzoyloxy-4-methyl-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)pentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate, 32. (White foam, 82%); R_f = 0.41 (1 : 9 MeOH–$CHCl_3$); $[\alpha]_D^{23}$ = −20.5 (*c* = 0.39, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 8.08 (d, *J* = 8.1 Hz, 2H), 8.01 (s, 1H), 7.59 (t, *J* = 7.3 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 2H), 7.30–7.16 (m, 5H), 6.90 (br s, 1H), 6.21 (br s, 1H), 6.15 (dd, *J* = 10.6, 3.1 Hz, 1H), 4.45–4.38 (m, 1H), 4.20–4.14 (m, 1H), 4.11–4.05 (m, 1H), 3.62 (s, 3H), 2.96 (dd, *J* = 13.6, 6.2 Hz, 1H), 2.87 (dd, *J* = 13.6, 6.9 Hz, 1H), 2.65–2.57 (m, 1H), 2.52–2.44 (m, 1H), 2.37–2.29 (m, 1H), 2.12 (s, 3H), 2.05–1.93 (m, 6H), 1.88–1.85 (m, 2H), 1.70–1.60 (m, 5H), 1.55–1.47 (m, 3H), 1.17 (d, *J* = 6.9 Hz, 3H), 0.95–0.86 (m, 12H); ^{13}C NMR (63 MHz, $CDCl_3$) *δ*: 176.6, 175.1, 170.8, 169.9, 165.4, 160.3, 150.1, 137.7, 133.5, 129.8, 129.4, 128.5, 128.3, 126.4, 123.4, 70.4, 69.7, 57.8, 51.7, 50.4, 48.5, 44.9, 41.2, 39.9, 37.8, 35.6, 35.5, 34.9, 31.8, 29.7, 25.2, 24.8, 23.2, 18.8, 17.7, 15.8, 10.7; LC/MS (ESI) *m*/*z* 790.3 $[M + H]^+$, 812.3 $[M + Na]^+$.

General procedure for the hydrolysis of methyl ester 24a–f and acetylation of 25a–d. To a solution of compound **24a** (120 mg, 0.17 mmol) in THF (5 mL), a 1 N aqueous solution of LiOH (510 μL, 0.51 mmol) was added. After stirring for 3 days, TFA (53 μL, 0.68 mmol) was then added and the solvent was removed *in vacuo* to give **25a**, which was used in the next step without further purification. To a solution of **25a** (126 mg, 0.16 mmol) in pyridine (3 mL), Ac_2O (1 mL) was added. The resulting mixture was stirred overnight. The mixture was concentrated under reduced pressure and the crude purified by FC.

(2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-Acetoxy-4-methyl-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)pentyl)-thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid, 1a. (White foam, 89% yield); R_f = 0.28 (1 : 9 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ = −1.42 (*c* 1.55, MeOH); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.07 (s, 1H), 7.21 (d, *J* = 4.2 Hz, 4H), 7.17–7.11 (m, 1H), 5.90 (dd, *J* = 10.8, 3.0 Hz, 1H), 4.39–4.32 (m, 1H), 4.21 (d, *J* = 8.2 Hz, 1H), 3.99–3.94 (m, 1H), 3.13–3.05 (m, 2H), 2.91(d, *J* = 6.8 Hz, 2H), 2.57–2.49 (m, 1H), 2.48–2.42 (m, 1H), 2.40 (s, 3H), 2.28–2.21 (m, 1H), 2.14 (s, 3H), 2.13–2.07 (m, 1H), 2.02–1.96 (m, 1H), 1.93–1.84 (m, 2H), 1.83–1.76 (m, 2H), 1.71–1.53 (m, 5H), 1.45–1.35 (m, 1H), 1.23–1.12 (m, 1H), 1.14 (d, *J* = 7.0 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.94–0.87 (m, 9H); ^{13}C NMR (100.5 MHz, CD_3OD) *δ*: 181.9, 173.7, 173.3, 171.7, 162.7, 151.1, 139.6, 130.5, 129.3, 127.3, 125.0, 71.3, 69.6, 59.6, 56.3, 52.0, 51.0, 44.0, 41.9, 39.2, 38.9, 38.1, 37.6, 33.7, 31.0, 25.9, 25.4, 23.5, 20.7, 19.5, 18.8, 18.6, 16.2, 11.1; LC/MS (ESI Ion Trap): *m*/*z* 720.3 $[M + H]^+$.

(2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-Acetoxy-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)-3-phenylpropyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid, 1b. (White foam, 80% yield); R_f = 0.28 (1 : 9 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ = −14.05 (*c* = 0.19, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) *δ* 8.16 (s, 1H), 7.45–7.16 (m, 10H), 6.11 (dd, *J* = 9.6, 3.9 Hz, 1H), 5.22 (dd, *J* = 9.8, 4.6 Hz, 1H), 4.50–4.38 (m, 1H), 4.32 (dd, *J* = 14.3, 8.6 Hz, 1H), 3.22–3.10 (m, 1H), 3.05–2.89 (m, 2H), 2.70 (ddd, *J* = 14.2, 10.3, 3.8 Hz, 1H), 2.62–2.40 (m, 3H), 2.35 (s, 3H), 2.21 (s, 3H), 2.16–1.31 (m, 12H), 1.20 (d, *J* = 6.9 Hz, 3H), 1.02 (d, *J* = 6.7 Hz, 3H), 0.97 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100.5 MHz, CD_3OD) *δ*: 182.5, 173.2, 171.8, 170.9, 163.0, 151.1, 143.1, 143.1, 139.7, 130.7, 129.9, 129.5, 128.8, 127.9, 127.6, 125.6, 72.5, 71.6, 70.9, 60.9, 57.8, 52.8, 52.1, 45.2, 44.0, 43.2, 40.6, 39.1, 32.2, 27.5, 26.6, 24.8, 22.2, 19.3, 17.7, 12.6; LC/MS (ESI) *m*/*z* 748.3 $[M + H]^+$, 770.3 $[M + Na]^+$.

(2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-Acetoxy-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)-3-(4-methoxyphenyl)propyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid, 1c. (White foam, 95% yield); R_f = 0.37 (1 : 9 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ = +21.63 (*c* = 0.59, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.13 (s, 1H), 7.29–7.03 (m, 7H), 6.85 (d, *J* = 8.6 Hz, 2H), 6.04 (dd, *J* = 9.7, 3.8 Hz, 1H), 5.12 (dd, *J* = 10.0, 4.8 Hz, 1H), 4.44–4.41 (m, 1H), 4.30–4.22 (m, 1H), 3.74 (s, 3H), 3.23 (t, *J* = 12.8 Hz, 1H), 2.86 (d, *J* = 6.1 Hz, 2H), 2.72–2.42 (m, 3H), 2.41 (s, 3H), 2.11 (s, 3H), 2.05–1.14 (m, 13H), 1.11 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.89 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100.5 MHz, CD_3OD) *δ*: 180.5, 179.3, 173.7, 172.4, 171.8, 164.0, 161.4, 151.4, 140.3, 135.8, 131.3, 130.1, 129.7, 128.3, 120.6, 115.9, 71.9, 69.8, 61.5, 57.1, 56.7, 51.7, 51.4, 44.4, 43.1, 42.6, 41.4, 40.7, 39.4, 31.3, 26.8, 25.6, 23.8, 21.6, 20.0, 17.0, 12.0; LC/MS (ESI) *m*/*z* 778.2 $[M + H]^+$, 800.2 $[M + Na]^+$.

(2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-Acetoxy-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)-3-cyclohexylpropyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid, 1d. (White foam, 75% yield); R_f = 0.38 (1 : 9 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ = +13.6 (*c* = 0.32, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 7.99 (s, 1H), 7.26–7.09 (m, 5H), 6.00 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.38 (d, *J* = 6.9 Hz, 1H), 4.24 (d, *J* = 8.2 Hz, 1H), 4.09–3.95 (m, 1H), 3.23–3.12 (m, 1H), 3.02–2.86 (m, 2H), 2.77–2.54 (m, 1H), 2.53–2.41 (m, 1H), 2.48 (s, 3H), 2.12 (s, 3H), 2.08–0.87 (m, 25H), 1.19 (d, *J* = 6.9 Hz, 3H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.91 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100.5 MHz, CD_3OD) *δ*: 173.9, 173.6, 172.2, 171.6, 163.9, 163.7, 151.9, 140.9, 131.5, 130.1, 128.1, 125.5, 73.3, 70.6, 61.8, 57.3, 52.4, 52.0, 45.0, 44.9, 43.5, 42.6, 40.0, 39.1, 38.7, 31.8, 31.6, 30.8, 29.7, 28.4, 28.2,

26.9, 26.0, 20.7, 20.4, 19.5, 17.1, 12.0; LC/MS (ESI) *m*/*z* 754.7 $[M + H]^+$, 776.7 $[M + Na]^+$.

(2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-Methoxy-4-methyl-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)pentyl)-thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid, 1e. (White foam, 40% yield); R_f = 0.47 (8 : 92 MeOH–$CHCl_3$); $[\alpha]_D^{23}$ = −7.3 (*c* = 0.24, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.08 (s, 1H), 7.21–7.14 (m, 5H), 4.48–4.42 (m, 1H), 4.37–4.33 (m, 1H), 4.22 (d, *J* = 8.5 Hz, 1H), 4.10–4.01 (m, 1H), 3.45 (s, 3H), 3.30–3.20 (m, 1H), 3.02–2.99 (m, 1H), 2.89 (d, *J* = 6.5 Hz, 2H), 2.42–2.34 (m, 2H), 2.37 (s, 3H), 2.01–1.87 (m, 5H), 1.80–1.56 (m, 7H), 1.43–1.37 (m, 1H), 1.26–1.20 (m, 1H), 1.12 (d, *J* = 6.9 Hz, 3H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.91 (m, 9H); ^{13}C NMR (100.5 MHz, CD_3OD) *δ*: 182.1, 175.8, 173.7, 173.6, 163.1, 151.1, 139.7, 130.6, 129.5, 127.6, 124.9, 79.9, 69.8, 59.8, 59.1, 56.4, 52.6, 50.7, 44.3, 42.7, 40.6, 39.3, 37.7, 33.8, 31.2, 26.1, 25.5, 23.6, 19.6, 18.7, 17.8, 16.5, 11.2; LC/MS (ESI) *m*/*z* 686.3 $[M + H]^+$, 708.3 $[M + Na]^+$.

(2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-Methoxymethyloxy-4-methyl-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)pentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid, 1f. (White foam, 57% yield); R_f = 0.43 (8 : 92 MeOH–$CHCl_3$); $[\alpha]_D^{23}$ = −18.4 (*c* = 0.20, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.13 (s, 1H), 7.18–7.09 (m, 5H), 4.83 (d, *J* = 9.7 Hz, 1H), 4.70 (d, *J* = 6.9 Hz), 1H, 4.65 (d, *J* = 6.9 Hz, 1H), 4.46–4.40 (m, 1H), 4.20 (d, *J* = 8.3 Hz, 1H), 4.06–4.00 (m, 1H), 3.11 (s, 3H), 3.26–3.23 (m, 1H), 2.89–2.84 (m, 2H), 2.69–2.63 (m, 1H), 2.53 (s, 3H), 2.45–2.41 (m, 1H), 2.20–1.89 (m, 5H), 1.84–1.45 (m, 9H), 1.27–1.19 (m, 1H), 1.11 (d, *J* = 6.6 Hz, 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.94–0.89 (m, 9H); ^{13}C NMR (100.5 MHz, CD_3OD) *δ*: 178.3, 176.5, 176.4, 174.8, 165.8, 153.5, 142.5, 133.3, 132.2, 130.3, 128.4, 100.6, 78.1, 72.0, 64.5, 62.8, 59.9, 59.2, 55.2, 46.6, 45.2, 40.4, 36.7, 35.7, 33.6, 28.8, 27.8, 26.6, 26.0, 22.3, 21.5, 19.2, 17.4, 14.1; LC/MS (ESI) *m*/*z* 716.2 $[M + H]^+$, 738.2 $[M + Na]^+$.

(2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-Benzoyloxy-4-methyl-3-((2*S*,3*S*)-3-methyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)pentyl)-thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid, 1g. To a solution of ester **32** (73 mg, 0.095 mmol) in DCE (3 mL), Me_3SnOH (11 mg, 0.58 mmol) was added and the reaction mixture was stirred under reflux for 32 h. The solvent was removed *in vacuo* and the residue was purified by FC (5 : 95 MeOH–CH_2Cl_2) to give **1g** (15 mg, 20%) as a white foam. R_f = 0.41 (1 : 9 MeOH–CH_2Cl_2); $[\alpha]_D^{23}$ = −26.6 (*c* = 0.33, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.11 (s, 1H), 8.09–7.68 (m, 5H), 7.24–7.20 (m, 5H), 6.21–6.18 (m, 1H), 4.40–4.34 (m, 1H), 4.09–4.06 (m, 1H), 3.16–3.10 (m, 1H), 2.98–2.91 (m, 3H), 2.60–2.56 (m, 1H), 2.48–2.43 (m, 2H), 2.39 (s, 3H), 2.30–2.24 (m, 1H), 2.05–1.98 (m, 1H), 1.93–1.85 (m, 3H), 1.75–1.53 (m, 7H), 1.41–1.30 (m, 2H), 1.18 (d, *J* = 7.2 Hz, 3H), 1.00–0.91 (m, 12H); ^{13}C NMR (100.5 MHz, CD_3OD) *δ*: 176.4, 174.5, 169.9, 165.8, 153.9, 142.4, 137.2, 133.8, 133.7, 133.5, 133.3, 132.7, 135.5, 132.2, 130.2, 127.8, 74.9, 72.8, 62.3, 59.3, 55.2, 53.9, 46.9, 44.8, 42.1, 41.4, 40.5, 36.7, 34.0, 28.7, 28.4, 26.5, 22.3, 21.6, 21.4, 19.2, 14.0; LC/MS (ESI) *m*/*z* 776.3 $[M + H]^+$, 798.3 $[M + Na]^+$.

Biological tests

Potential anti-tumor activity was evaluated through *in vitro* assays based on the determination of cytotoxicity of the compounds towards the HT29 (Human Caucasian Colon Adenocarcinoma) cell line. HT29 cells from ECACC (European Collection of Cell Cultures) were purchased from Sigma Aldrich, Milan, Italy.

The assays were performed according to the previously reported procedure. The cell line was grown in 75 cm^2 flasks with culture medium DMEM (Dulbecco's Medium Eagle Modified, Sigma Aldrich) and the following additives (Sigma Aldrich): L-glutamine 2 mM, 10% fetal bovine serum, penicillin/streptomycin, fungizone, gentamycin. The incubation was carried out in a modified atmosphere incubator (37 °C, 5% CO_2). When cell confluence was obtained, the cells were propagated by dilution in a ratio 1 : 3/1 : 10 by using a 0.25% trypsin solution and then transferred into 96 well plates in the suitable culture medium. The cells were then treated for 72 hours with different doses of the compounds under examination. For these assays, the compounds were solubilised in DMSO. All the tests were carried out at a constant DMSO concentration equal to 0.1% by weight.

To control the cellular viability, the ATPlite test (Perkin Elmer) was used, based on the determination of the ATP production. ATP is in fact a marker of cellular viability as it is present in all the metabolically active cells. The test is based on the chemiluminescence due to the ATP reaction with the luciferase and the D-luciferin. The emitted light is proportional to the ATP concentration. The tests were carried out by a Victor 3 instrument (Perkin Elmer).

IC_{50} values were obtained from four different experiments.

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