Total synthesis and evaluation of Wnt signal inhibition of melleumin A and B, and their derivatives†

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The total synthesis of melleumin A (**1**), a novel cyclic depsipeptide isolated from the myxomycete *Physarum melleum*, and 3-*epi*-melleumin A (**6**) was achieved. Melleumin A-like compounds were also designed and synthesized; analysis of these melleumin A-like compounds showed moderate Wnt signal inhibition. Comparison of the inhibition activity of melleumin B and its three epimers, melleumin A, 3-*epi*-melleumin A and three melleumin A-like compounds led to further investigation of the structural conformation of the active molecules. The scaffold of melleumin is a potential target in the search for "peptide-like" Wnt signaling inhibitors.

Introduction

Isolation of new natural products and the synthesis of small molecules based on their scaffolds is of chemical relevance to living cells and organs, implicated in the binding to proteins, absorption, distribution, metabolism, and excretion.[1] Recently, we have been attempting natural product isolation by guided bioactivity, such as Wnt signal inhibition,[2] Hh signal inhibition,[3] and TRAIL resistance overcoming activity.[4] Wnt signaling shows aberrant activation in many cancer cells, especially in colon cancers.[5] The aberrant activation of this signal is caused by mutation or loss of function in β-catenin, T-cell factor/lymphoid enhancer factor (TCF/LEF), and glycogen synthase kinase 3β (GSK3β) and other things. Small molecules that act on certain molecular components in the Wnt signal pathway would be potential candidates for treating cancer. Several Wnt signal inhibitors have been previously reported.[6] However, there are not many effective Wnt signal inhibitors. We have also reported natural products or their derivatives as Wnt signal inhibitors, such as bisindole alkaloid *cis*-dihydroarcyriarubin C,[2a] melleumin derivatives,[2b,c] and new natural products eleutherinoside B–E.[2d]

Novel peptide lactone, melleumin A (**1**), and its *seco* acid methyl ester, melleumin B (**2**) were discovered by our group in 2005 from cultured plasmodium of the myxomycete *Physarum melleum* (Fig. 1).[7] Stereochemistry of **1** and **2** at the chiral centers was established as 3*S*, 10*S*, and 11*R*-configurations. The absolute stereochemistry of the C-4 position was determined as *S* by total synthesis of melleumin B (**2**).[2b] Because the quantity of isolated **1** and **2** was very small, we decided to synthesize those compounds to evaluate biological activity. After synthesizing **2** and its related isomers, we found Wnt signal inhibitory activities in the isomers of **2** using a cell-based assay. The 10*R*-epimer (**3**), 3*R*-epimer (**4**) and (3*R*, 10*R*)-epimer (**5**) of **2** showed moderate activity.[2b,c] Luo *et al.* reported the first total synthesis of **1**, 4*R*-epimer and other derivatives by intramolecular lactamization at the N5–C6 amide bond[8] and found moderate Wnt inhibition in 4-*epi*-melleumin B and 4-*epi*-deoxymelleumin A, but not in melleumin A (**1**) and 4-*epi*-melleumin A. In this report, we describe the total synthesis of melleumin A (**1**) and its 3*R*-epimer (**6**). In order to analyze Wnt inhibitory activities on cyclic compounds related to **1**, melleumin A-like compounds that do not contain a lactone subunit that is easily cleaved in cells were designed and synthesized. These compounds showed moderate Wnt inhibitory activities.

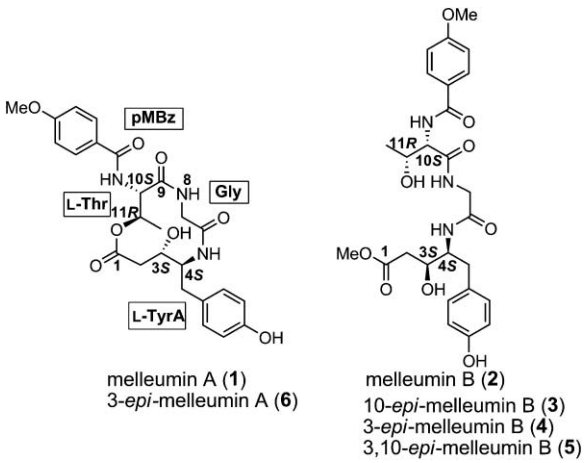

Fig. 1 Structure of melleumin A, B and derivatives.

Results and discussion

Total synthesis of mellumin A (1) and 3-*epi*-melleumin A (6)

Melleumin A (**1**) and B (**2**) consist of four residues: *p*-methoxybenzoic acid (pMBz), L-threonine (L-Thr), glycine (Gly), and an unusual amino acid, a tyrosine-attached acetic acid (TyrA). Because compound **2** is a *seco* acid methyl ester of **1**, we attempted intramolecular lactone formation of the *seco* acid of **1** that was obtained by hydrolysis of synthesized **2**. However, several conditions prevented cyclization.[9] Therefore, we changed strategies to synthesize **1** by N8–C9 amide bond formation as a cyclization step (Scheme 1). Compound **7** could be prepared from dipeptide **8** (TyrA-Gly unit) and *N*-*p*-methoxybenzoyl-L-threonine

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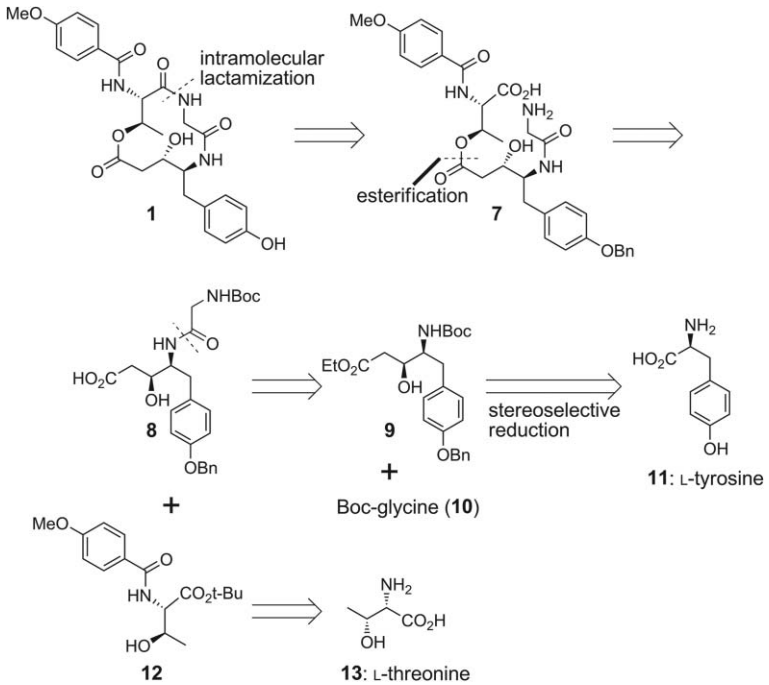
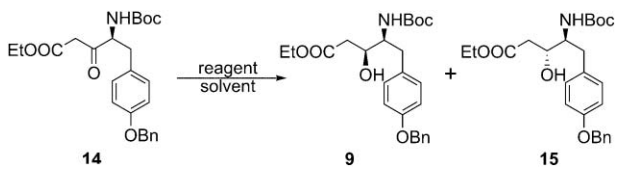


Scheme 1 Retrosynthetic approach to melleumin A (**1**).

Table 1 Streoselective synthesis of TyrA unit



Entry	Reagent (eq.)	Solvent	T/°C	Time/h	Ratio[a] **9**:**15**	Yield (%)
1	K-selectride (2.4)	THF	0	9	93:7	69
2	$NaBH_4$ (1.5)	EtOH	−78	1	9:91	94

[a] The ratio was determined by HPLC.

12 (pMBz-L-Thr unit). Dipeptide **8** could be obtained from (3*S*,4*S*)-β-hydroxy ester **9** and Boc-glycine (**10**).

To obtain (3*S*,4*S*)-TyrA unit **9** stereoselectively, the reduction of β-ketoester **14** was carried out (Table 1).[2c] β-Ketoester **14** was synthesized from *N*-Boc-*O*-phenylmethyl-L-tyrosine. As shown in Table 1, desired (3*S*,4*S*)-β-hydroxy ester **9** was obtained in 69% yield with a ratio of **9**:**15** = 93:7, with K-selectride. When $NaBH_4$ was used, (3*R*,4*S*)-TyrA unit **15** was predominately obtained as expected, which was used for the synthesis of 3-*epi*-melleumin A (**6**).

After deprotection of the Boc group, coupling with *N*-Boc-glycine gave **16** in good yield (Scheme 2). Compound **16** was hydrolyzed by lithium hydroxide to give a desired carboxylic acid that was used in the next step without further purification. Compound **12** (*p*MBz-L-Thr unit), synthesized from L-threonine *tert*-butyl ester hydrochloride and *p*-methoxybenzoyl chloride, and the aforementioned carboxylic acid were coupled using DCC to give compound **18** in 50% yield in 2 steps. EDC gave comparable yield with DCC, but 2-methyl-6-nitrobenzoic anhydride (MNBA)[10] reagent provided **18** in low yield. The Boc group and *tert*-butyl ester of **18** were deprotected to give compound **7**. Macrolactamization of **7** was investigated for various coupling reagents. HBTU, TBTU, PyBOP, and DEPBT were unsuccessful, but FDPP and HATU provided **20** in 28% and 24% yield (2 steps), respectively. The benzyl group was finally removed by hydrogenolysis to give melleumin A (**1**). The ^1H and ^{13}C NMR spectral data of the synthetic material were identical to those of the natural product.[1] The optical rotation of synthetic melleumin A (**1**) showed the same sense as the natural product {$[\alpha]_D^{18}$ +16 (*c* 0.97, MeOH); $[\alpha]_D^{18}$ +27 (*c* 0.15, MeOH) lit.[7]}. X-Ray crystallographic analysis of synthetic melleumin A (**1**) was successful as shown in Fig. 2.[11] This result strongly confirmed the absolute structure of melleumin A (**1**). Starting from (3*R*,4*S*)-β-hydroxy ester **15**, 3-*epi*-melleumin A (**6**) was also synthesized as shown in Scheme 2. To

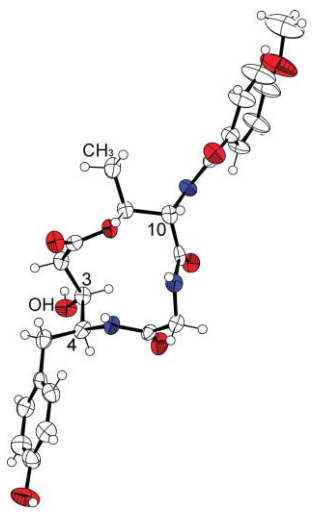
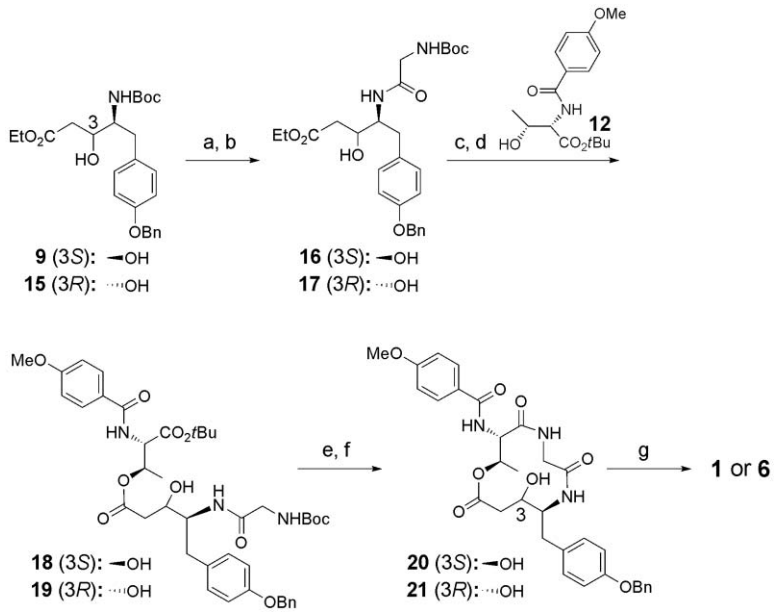


Fig. 2 X-Ray structure of melleumin A (**1**).



Scheme 2 Synthesis of melleumin A (**1**) and 3-*epi*-melleumin A (**6**). *Reagents and conditions*: (a) TFA or HCl; (b) Boc-Gly, EDC, HOBt, DMAP, 80% (**16** (3*S*), 2 steps from **9**), 61% (**17** (3*R*), 2 steps from **15**); (c) LiOH, 0 °C; (d) **12**, DCC, DMAP, 50% (**18** (3*S*), 2 steps from **16**), 36% (**19** (3*R*), 2 steps from **17**); (e) TFA; (f) FDPP, 28% (**20** (3*S*), 2 steps from **18**), 22% (**21** (3*R*), 2 steps from **19**); (g) H_2, 10% Pd/C, 97% (**1**), 77% (**6**).

ensure the absolute configuration at C-10 of **6**, the hydrolysate of **6** with 6 N HCl was analyzed by chiral TLC with authentic samples: L-threonine (10*S*) and D-allo-threonine (10*R*). The R_f value of hydrolysate was identical to that of L-threonine.[12]

Synthesis of mellumin A-like compounds

Next, we examined the biological activity of melleumin A (**1**) and 3-*epi*-melleumin A (**6**). Unfortunately, melleumin A (**1**) had weak Wnt signal inhibitory activity and 3-*epi*-melleumin A (**6**) had no inhibitory activity. However, we had previously explored and found that melleumin B derivatives became Wnt signal inhibitors.[2b,c] Therefore, we thought it possible to add Wnt inhibition to melleumin A-like compounds. The successful synthesis and results of moderate inhibition by 4-*epi*-deoxymelleumin A reported by Luo *et al.* also encouraged us to attempt the synthesis of melleumin A-like compounds. We designed melleumin A-like compounds as shown in Fig. 3. Although the target molecule of active melleumins is unknown at this stage, most melleumins show moderate or weak Wnt inhibition. Therefore we hypothesized that two amides in the ring and aryl groups could be important as a common structure (Fig. 3, same moieties as melleumin A). Because melleumin B derivatives, which are open chain analogues

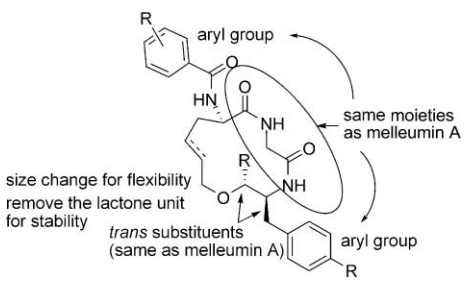


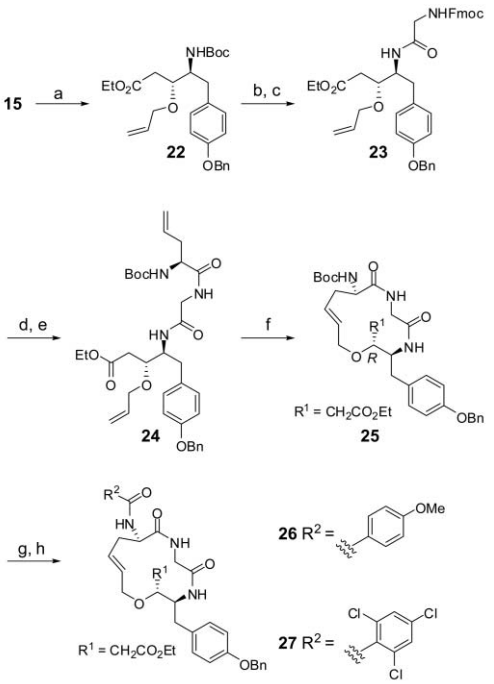
Fig. 3 Design of melleumin A-like compounds.

of melleumin A, have moderate Wnt inhibitory activity, greater flexibility would be important to fit the target molecule (larger ring). The lactone moiety was removed for compound stability in cells. At this time, a 13-membered ring was chosen instead of the 12-membered ring of melleumin A.

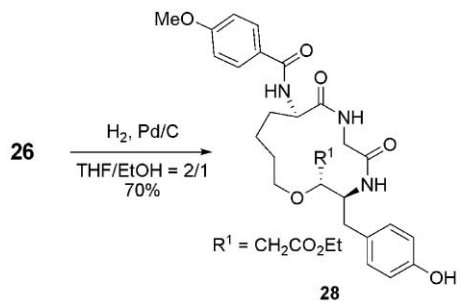
Because the allyl ether moiety at C-3 is used for cyclization, compound **15** (3*R*) was used to maintain the stereochemistry of **1**; substitution on the ring at C-3 and C-4 was predicted as *trans*. Synthesis of melleumin A-like compounds is shown in Scheme 3. Allylation of **15** by Pd catalyst gave compound **22** in 96% yield. Deprotection of the Boc group followed by coupling with glycine gave **23** in good yield. Coupling reaction with *N*-Boc-L-allylglycine[13] provided compound **24** in 83% yield in 2 steps. Ring-closing metathesis (RCM) reaction was selected for construction of the ring system.[14] RCM by second generation Grubbs' catalyst proceeded smoothly to give cyclized 13-membered ring **25** in 98% yield in 20 min. When Grubbs' catalyst (bis(tricyclohexylphosphine)benzylidine ruthenium(IV) dichloride) was used, **25** was obtained in 67% yield under reflux conditions in CH_2Cl_2 for 24 h. The yield with second generation Grubbs' catalyst at 80 °C in toluene decreased to 82%. The Boc group of **25** was removed, then two acyl chlorides gave each derivative (**26**, **27**). Structural elucidation of **26** was performed by H–H COSY, HMBC and HMQC (see the ESI†). Compound **26** exhibited the same substitution as melleumin A (**1**), a *p*-methoxybenzoyl unit. Compound **27** has a greater electron withdrawing acyl unit, a 2,4,6-trichlorobenzoyl unit. We also synthesized compound **28** from **26** by hydrogenation (Scheme 4).

Wnt signal inhibitory activity

Next we examined Wnt signal inhibitory activity of the synthesized compounds using a luciferase reporter gene assay. Wnt signaling activates gene transcription with a complex between



Scheme 3 Synthesis of melleumin A-like compounds. *Reagents and conditions*: (a) allyl ethyl carbonate, $Pd_2(dba)_3$, dppb, THF, 65 °C, 96%; (b) TFA; (c) Fmoc-glycine, PyBOP, HOBt, DIPEA, 61% (2 steps); (d) piperidine; (e) *N*-Boc-L-allyglycine, PyBOP, HOBt, iPr_2NEt, 83% (2 steps); (f) 2nd Grubbs' cat. (20 mol%), CH_2Cl_2, reflux, 98%; (g) TFA; (h) *p*-methoxy benzoyl chloride, (2,4,6-trichlorobenzoyl chloride for **27**), TEA, 54% (**26**), 53% (**27**) (2 steps).



Scheme 4 Synthesis of melleumin A-like compounds.

β-catenin and TCF/LEF, a DNA-binding protein. Super TOP-Flash,[15] a reporter plasmid with multiple TCF-binding sites (CCTTTGATC), was stably transfected into 293 cells. Super FOP-Flash has eight mutated TCF-binding sites (CCTTTGGCC); therefore, a selective inhibitor would not affect transcription in Super FOP-Flash-transfected cells. We tested Super FOP-Flash activity on all samples. Because it is also possible that a decrease of luciferase reporter activity in this assay may be due to cytotoxicity of the test samples, we also examined the cytotoxicity of samples using fluorometric microculture cytotoxicity assay.[16] Therefore, compounds exhibiting both low luciferase reporter activity and high cell viability are to be considered "potent" compounds. Results are shown in Fig. 4, along with cell viability.

Melleumin A (**1**) showed weak inhibitory activity. We evaluated three newly synthesized melleumin A-like compounds (**26–28**). Interestingly, all compounds showed moderate inhibitory activities. Of them, compound **26**, exhibited the strongest activity in a dose-dependent manner with high cell viability. Comparing the activities of **26** and **28**, the Bn unit and C–C double bond seemed to be critical. From these results, modification of the melleumin A scaffold is effective to construct cyclic peptides like Wnt inhibitors.

Fig. 5 shows the comparison of Wnt signal inhibitory activities of melleumin A, B, their derivatives and melleumin A-like compounds (50 μM). Natural products, melleumin A (**1**) and melleumin B (**2**), reduced Wnt transcriptional activity to 74% and 64%, respectively. Of them, 10-*epi*-melleumin B (**3**) seemed to be the most active (46%). Regarding cyclic peptide type (melleumin A type) compounds, 3-*epi*-melleumin A (**6**) lost activity; however, melleumin A-like compound (**26**) showed 20% more inhibition (54%) compared with **1** (74%).

To evaluate the structural differences between melleumin A (**1**) and melleumin A-like compounds, DFT calculation of **26**, the most active of the three melleumin A-like compounds, was carried out (Fig. 6). The DFT calculation at the level of B3LYP/6-31G* suggested a slight difference in the three-dimensional orientation of two amide units in the ring. The two amide torsion angle (ϕ) was changed to 86.7° (**26**) from 112.4° (**1**). The distances of N1–N2 and N1–N3 were changed to 3.35 Å (**26**) from 2.93 Å (**1**), and 3.64 Å (**26**) from 3.71 Å (**1**), respectively. Since two amides in the ring and aryl groups are common structures in bioactive melleumins, Wnt inhibition of **26** might be due to this conformational change in the structure.

Conclusion

The total synthesis of melleumin A (**1**), 3-*epi*-melleumin A (**6**) and X-ray crystal analysis of synthesized compound **1** were achieved. Moreover, we designed and synthesized melleumin A-like compounds (**26–28**) to seek a possible Wnt signaling inhibitor. We succeeded to add the Wnt inhibitory activity of a melleumin A-like compound, though natural product **1** showed weak activity. Since few small molecules are known as Wnt signal inhibitors and their clinical use has received great attention, we believe that peptide-based inhibitors will be worth synthesizing to evaluate their potential. Synthesis of melleumin A-like compounds and application using solid-phase reactions to construct small molecule libraries based on melleumin A-like compounds are in progress.

Experimental

General

Optical rotations were measured with a JASCO P-1020 polarimeter. IR spectra were measured on ATR on a JASCO FT-IR 230 spectrophotometer. NMR spectra were recorded on JEOL A 400 and A 500 spectrometers with deuterated solvents, the chemical shift of which was used as an internal standard. FABMS was measured on a JEOL JMS-AX500 and HR-FABMS using a JEOL HX-110A spectrometer. HREIMS was measured on a JEOL JMS-GC Mate and HR-ESIMS using an Exactive spectrometer.

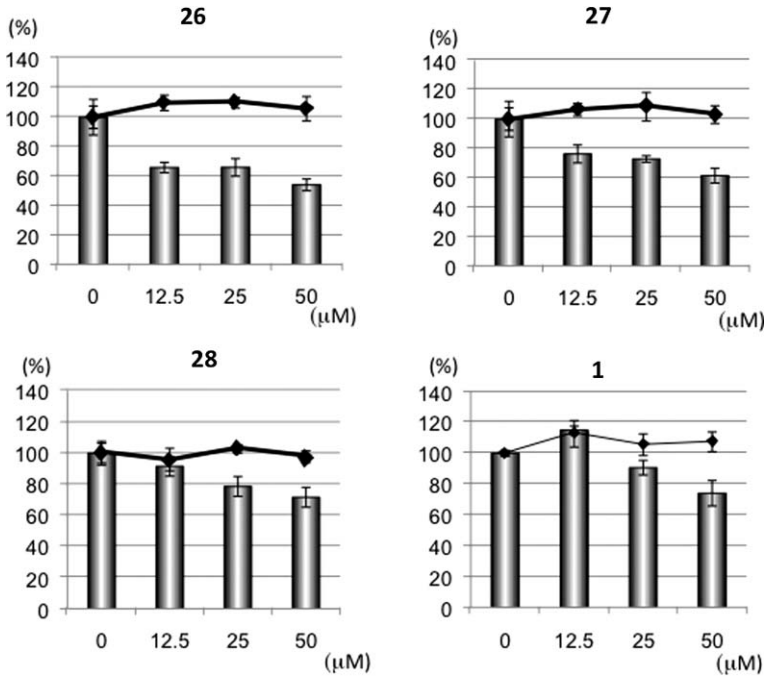


Fig. 4 Wnt signal inhibitory activities of melleumin A (**1**) and melleumin A-like compounds (**26**, **27**, **28**). Fold activation of Super TOP-Flash (solid columns) and cell viability (solid curves). STF/293 cells (a 293 human embryonic kidney cell line stably transfected with Super Top-Flash, 3×10^4) were split into 96-well plates and 24 h later cells were treated with 15 mM LiCl and testing samples (DMSO solution). Super FOP-Flash activities at each concentration were not affected (data not shown). $N = 3$, Bars = sd.

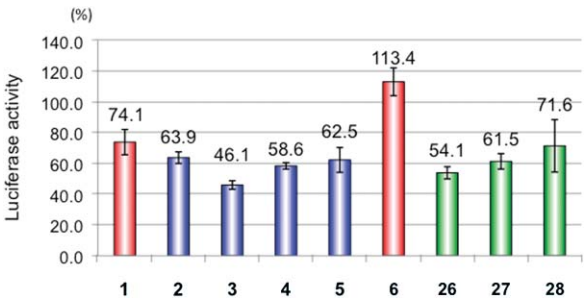


Fig. 5 Comparison of Wnt signal inhibitory activities of melleumin A, B, their derivatives and melleumin A-like compounds at 50 μM. Fold activation of Super TOP-Flash (solid columns). STF/293 cells (3×10^4) were split into 96-well plates and 24 h later cells were treated with 15 mM LiCl and testing samples (DMSO solution). $N = 3$, Bars = sd. The activity of the untreated cells was defined as 100%.

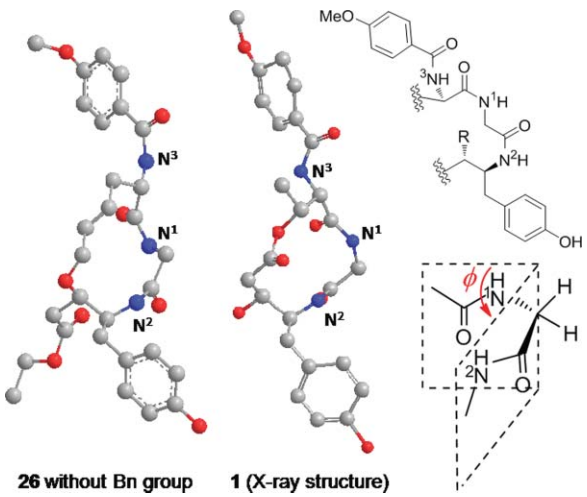


Fig. 6 Structural comparison of **26** (without Bn) and **1**. Hydrogen atoms have been omitted for clarity.

(3*S*,4*S*)-Ethyl-5-(4-(benzyloxy)phenyl)-4-(2-(*tert*-butoxycarbonylamino)acetamido)-3-hydroxypentanoate (16). A solution of **9** (940.8 mg, 2.12 mmol) in CH_2Cl_2 (10.5 mL) was treated with TFA (10.5 mL) under argon atmosphere. The reaction mixture stirred for 30 min. The solvent was removed *in vacuo*, the residue was used without further purification in the following reaction. A solution of *N*-Boc-glycine (757 mg, 2.55 mmol), EDC·HCl (488 mg, 2.55 mmol), HOBt·H_2O (344 mg, 2.55 mmol) and DMAP (311 mg, 2.55 mmol) in CH_2Cl_2 (11.0 mL) was stirred for 10 min at 0 °C under argon atmosphere. To the mixture was added a solution of the deprotected material obtained above in CH_2Cl_2 (11.0 mL). After stirring overnight at RT, the reaction mixture was diluted with ethyl acetate, washed successively with water, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–ethyl acetate = 1/1) to afford **16** (850 mg, 80%, 2 steps from **9**) as a pale yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, J = 7.1 Hz, 3H), 1.46 (s, 9H), 2.33 (dd, J = 2.4, 17.1 Hz, 1H), 2.51 (dd, J = 17.1, 10.5 Hz, 1H), 2.83 (dd, J = 13.7, 7.0 Hz, 1H), 2.88 (dd, J = 13.7, 8.7 Hz, 1H), 3.60 (br s, 1H), 3.73 (dd, J = 16.8, 5.7 Hz, 1H), 3.78 (dd, J = 16.8, 6.2 Hz, 1H), 3.99–4.16 (m, 4H), 5.03 (s, 2H), 5.06 (br s, 1H), 6.46 (d, J = 9.0 Hz, 1H), 6.91 (d, J = 8.7 Hz, 2H), 7.16 (d, J = 8.7 Hz, 2H) and 7.29–7.44 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 28.2, 37.1, 38.5, 44.2, 54.0, 60.7, 66.8, 69.8, 80.1, 114.7, 127.4, 127.8, 128.4, 130.0, 130.2, 136.9, 156.0, 137.4, 169.5, and 173.2; HRMS (FAB) $[M+H]^+$: calcd

for $C_{27}H_{37}N_2O_7$ 501.2601, found 501.2642; IR (ATR); 3321, 2979, 2926, 1712, 1654, 1509, 1239, 1161, 1022, 735 and 696 cm^{-1}; $[\alpha]_D^{22}$ –39.8 (*c* 3.0, $CHCl_3$).

(2*S*,3*R*)-3-Hydroxy-2-(4-methoxybenzamido)butanoic *tert*-butyl ester (12). A solution of L-threonine-*tert*-butyl ester hydrochloride (334 mg, 1.58 mmol) and triethylamine (0.55 mL, 3.94 mmol) in CH_2Cl_2 (6.3 mL) was treated with 4-methoxybenzoyl chloride (0.24 mL, 1.73 mmol) at 0 °C under argon atmosphere. After stirring overnight at RT, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–ethyl acetate = 2/1) to afford **12** (485 mg, 99%) as a pale yellow amorphous solid. ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (d, J = 5.3 Hz, 2H), 1.48 (9H, s), 3.80 (3H, s), 3.88 (1H, br s), 4.37 (1H, br d, J = 4.4 Hz), 4.66 (1H, dd, J = 8.8, 1.2 Hz), 6.85 (2H, d, J = 8.6 Hz), 7.20 (1H, d, J = 8.7 Hz) and 7.80 (2H, d, J = 8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.1, 28.0, 55.3, 58.2, 68.6, 82.4, 113.6, 126.0, 129.0, 162.3, 167.4 and 170.3; IR (ATR) 3394, 2980, 2935, 1733, 1630, 1606, 1537, 1255, 1086, 839 and 764 cm^{-1}; HRMS (FAB) $[M+H]^+$: calcd for $C_{16}H_{24}NO_5$ 310.1654, found 310.1656. IR (ATR); 3394, 2980, 2935, 1733, 1629, 1606, 1606, 1537, 1506, 1349, 1255, 1159, 1123, 1085, 1011, 838 and 764 cm^{-1}; $[\alpha]_D^{20}$ +32.2 (*c* 3.0, $CHCl_3$).

(3*S*,4*S*)-((2*R*,3*S*)-4-*tert*-Butoxy-3-(4-methoxybenzamido)-4-oxobutan-2-yl)-5-(4-(benzyloxy)phenyl)-4-(2-(*tert*-butoxycarbonylamino)acetamido)-3-hydroxypentanoate (18). To a solution of **16** (412 mg, 0.823 mmol) in solvent (THF–H_2O = 1/1) was added LiOH·H_2O (86 mg, 2.06 mmol) at 0 °C, and the mixture was stirred for 2.5 h. The mixture was acidified with 2 N HCl, and then extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*, the residue was used without further purification in the following reaction. The residue was dissolved in CH_2Cl_2 (3.9 mL). To this solution DCC (131 mg, 0.633 mmol), DMAP (28 mg, 0.231 mmol) and a solution of **12** (310 mg, 1.00 mmol) in CH_2Cl_2 (3.9 mL) were added. After stirring for 48 h at RT, the reaction mixture was quenched with 10% aqueous $KHSO_4$ and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–ethyl acetate = 2/1 to 1/1) to afford **18** (257 mg, 44%) as a colorless amorphous solid. ^1H NMR (400 MHz, $CDCl_3$) δ 1.29 (d, J = 6.4 Hz, 3H), 1.43 (s, 9H), 1.44 (s, 9H), 2.34 (dd, J = 15.6, 3.0 Hz. 1H), 2.48 (dd, J = 15.6, 9.9 Hz, 1H), 2.81 (dd, J = 13.4, 6.8 Hz, 1H), 2.87 (dd, J = 13.4, 8.4 Hz, 1H), 3.65 (br s, 1H), 3.78 (d, J = 5.7 Hz, 2H), 3.85 (s, 3H), 3.97 (br d, J = 9.9 Hz, 1H), 4.12 (q, J = 8.4 Hz, 2H), 4.88 (dd, J = 9.0, 2.6 Hz, 1H), 5.02 (s, 2H), 5.18 (br s, 1H), 5.52 (qd, J = 6.4, 2.6 Hz, 1H), 6.47 (d, J = 9.5 Hz, 1H), 6.87 (d, J = 8.6 Hz, 2H), 6.96 (d, J = 8.5 Hz, 2H), 7.13 (d, J = 8.5 Hz, 2H), 7.30–7.44 (m, 5H), 7.86 (d, J = 8.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1, 27.8 (3C), 28.2 (3C), 37.3, 39.5, 54.1, 55.4, 56.2, 67.1, 6.9, 71.3, 80.3, 83.1, 113.8 (2C), 114.9 (2C), 125.8, 127.5 (2C), 127.9, 128.5 (2C), 129.2 (2C), 129.8, 130.3 (2C), 136.9, 157.5, 162.5, 167.2, 169.4, 169.5 and 171.7; HRMS (FAB) $[M+H]^+$: calcd for $C_{41}H_{54}N_3O_{11}$ 764.3758, found 764.3794; IR (ATR); 3309, 2970, 2935, 1737, 1653, 1507, 1231, 1155, 1026, 844 and 696 cm^{-1}; $[\alpha]_D^{21}$ +9.9 (*c* 3.0, $CHCl_3$).

***N*-((2*R*,3*S*,9*S*,10*S*)-9-(4-(Benzyloxy)benzyl)-10-hydroxy-2-methyl-4,7,12-trioxo-1-oxa-5,8-diazacyclododecan-3-yl)-4-methoxybenzamide (20).** A solution of **18** (136 mg, 0.178 mmol) in CH_2Cl_2 (3.6 mL) was treated with TFA (0.89 mL) at 0 °C under argon atmosphere. After stirring for 4 h, the solvent was removed *in vacuo*, the residue was used without further purification in the following reaction. To a solution of the residue and $^iPr_2NEt$ (0.15 mL, 0.89 mmol) in CH_2Cl_2 (89 mL) was added FDPP (136 mg, 0.356 mmol) at 0 °C. After stirring overnight at RT, the reaction mixture was concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–ethyl acetate = 1/1 to 0/1) to afford **20** (24 mg, 23%) as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ 1.21 (3H, d, J = 6.4 Hz), 2.36 (1H, t, J = 11.6 Hz), 2.57 (1H, dd, J = 12.5, 5.0 Hz), 2.64 (1H, dd, J = 14.2, 11.6 Hz), 2.91 (2H, br d, J = 12.6 Hz), 3.44–3.49 (1H, m), 3.72–3.78 (1H, m), 3.81 (3H, s), 4.11–4.18 (1H, m), 4.98–5.03 (1H, m), 5.04 (2H, s), 5.47 (1H, d, J = 4.4 Hz), 5.61–5.65 (1H, m), 6.23 (1H, d, J = 10.1 Hz), 6.90 (2H, d, J = 8.7 Hz), 7.00 (2H, d, J = 9.0 Hz), 7.10 (2H, d, J = 8.7 Hz), 7.29–7.44 (5H, m), 7.91 (2H, d, J = 9.0 Hz), 8.09 (1H, br d, J = 8.8 Hz) and 8.47 (1H, br t, J = 5.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ = 4/1) δ 16.1, 29.4, 31.6, 36.9, 44.6, 55.0, 55.1, 55.9, 68.6, 69.8, 72.3, 113.6 (2C), 114.5 (2C), 125.1, 127.2n, 127.7, 128.3 (2C), 129.0 (2C), 129.6 (2C), 130.4, 136.8, 157.1, 162.6, 167.9, 169.8, 170.4 and 172.0; HRMS (FAB) $[M+H]^+$: calcd for $C_{32}H_{36}N_3O_8$ 590.2502, found 590.2518; IR (ATR); 3319, 2970, 2926, 1737, 1651, 1509, 1366, 1250, 1076, 1027, 844, 735 and 696 cm^{-1}; $[\alpha]_D^{21}$ +19.9 (*c* 0.25, $CHCl_3$–MeOH = 1/1).

Melleumin A (1). To a solution of **20** (18 mg, 31 μmol) in solvent (EtOAc–EtOH = 1/2) was added 25% palladium on carbon (4 mg). The reaction mixture was stirred for 24 h under hydrogen and then filtered through Celite and concentrated *in vacuo*. The crude product was purified by preparative TLC (silica gel, MeOH–$CHCl_3$ = 1/10), to give melleumin A (15 mg, 97%) as a white solid. For X-ray analysis, the compound was further purified by HPLC (Develosil C30, 25% CH_3CN, 2.5 ml min^{-1}, 32 min). ^1H NMR (400 MHz, DMSO-d_6) δ 1.20 (3H, d, J = 6.2 Hz), 2.35 (1H, t, J = 11.7 Hz), 2.53–2.61 (2H, m), 2.85 (1H, dd, J = 14.2, 11.9 Hz), 3.37–3.49 (3H, m), 3.71–3.77 (1H, m), 3.81 (3H, s), 4.08–4.13 (1H, m), 4.99 (1H, dd, J = 9.0, 3.5 Hz), 5.45 (1H, br s), 5.52 (1H, qd, J = 6.5, 3.7 Hz), 6.26 (1H, d, J = 9.9 Hz), 6.63 (2H, d, J = 8.4 Hz), 6.96 (2H, d, J = 8.4 Hz), 7.00 (2H, d, J = 9.0 Hz), 7.91 (2H, d, J = 9.0 Hz) 8.09 (1H, br d, J = 9.0 Hz) and 8.63 (1H, brs); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.1, 30.8, 38.7, 44.4, 54.9, 55.1, 69.5, 71.7, 113.4, 114.9, 126.0, 129.4, 129.5, 155.4, 161.8, 166.6, 169.1, 169.2 and 170.7; HRMS (FAB) $[M+H]^+$: calcd for $C_{25}H_{30}N_3O_8$ 500.2033, found 500.2030; IR (ATR); 3369, 3031, 2943, 1739, 1635, 1507, 1438, 1365, 1229, 1217 and 1109 cm^{-1}; $[\alpha]_D^{18}$ +16 (*c* 0.97, MeOH).

(3*R*,4*S*)-Ethyl-5-(4-(benzyloxy)phenyl)-4-(2-(*tert*-butoxycarbonylamino)acetamido)-3-hydroxypentanoate (17). ^1H NMR (500 MHz, $CDCl_3$) δ 1.25 (3H, t, J = 7.1 Hz), 1.43 (9H, s), 2.46–2.55 (2H, m), 2.78 (1H, dd, J = 14.3, 8.9 Hz), 2.90 (1H, dd, J = 14.3, 4.6 Hz), 3.62 (1H, dd, J = 16.3, 5.6 Hz), 3.67 (1H, br dd, J = 16.3, 6.0 Hz), 3.92 (1H, br d, J = 4.0 Hz), 4.03–4.07 (1H, m), 4.12–4.18 (1H, m),3.78 (1H, q, J = 7.1 Hz), 5.03 (2H, s), 5.16 (1H, br t, J = 5.7 Hz), 6.46 (1H, d, J = 8.9 Hz), 6.89 (2H, d, J = 8.5 Hz), 7.11 (2H, d, J = 8.5 Hz) and 7.29–7.43 (5H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 28.2 (3C), 34.3, 38.1, 44.3, 54.2, 60.8, 69.6,

69.9, 80.2, 114.8 (2C), 127.4 (2C), 127.9, 128.5 (2C), 129.5, 130.2 (2C), 136.9, 156.1, 157.4, 169.6 and 172.7; HRMS (FAB) $(M)^+$: calcd for $C_{27}H_{36}N_2O_7$ 500.2522, found 500.2497; IR (ATR); 3342, 3281, 1732, 1685, 1654, 1541, 1508, 1365, 1218, 1165, 1018 and 696 cm^{-1}; $[\alpha]_D^{19}$ −7.7 (*c* 3.0, $CHCl_3$).

(3*R*,4*S*)-((2*R*,3*R*)-4-*tert*-Butoxy-3-(4-methoxybenzamido)-4-oxobutan-2-yl)-5-(4-(benzyloxy)phenyl)-4-(2-(*tert*-butoxycarbonylamino)acetamido)-3-hydroxypentanoate (19).

^1H NMR (400 MHz, $CDCl_3$) δ 1.34 (3H, d, J = 6.6 Hz), 1.43 (9H, s), 1.45 (9H, s), 2.51 (2H, d, J = 6.5 Hz), 2.79 (1H, dd, J = 14.2, 8.7 Hz), 2.88 (1H, dd, J = 14.2, 4.6 Hz), 3.65 (2H, br d, J = 5.9 Hz), 3.72 (1H, br t, J = 4.9 Hz), 3.84 (3H, s), 3.97 (1H, br d, J = 9.9 Hz), 4.01–4.07 (1H, m), 4.12–4.20 (1H, m), 4.91 (1H, dd, J = 9.0, 2.9 Hz), 5.02 (2H, s), 5.55 (1H, qd, J = 6.6, 2.9 Hz), 6.15–6.24 (1H, m), 6.15–6.24 (1H, m), 6.85–6.89 (1H, m), 6.89 (2H, d, J = 8.7 Hz), 6.95 (2H, d, J = 8.9 Hz), 7.09 (2H, d, J = 8.9 Hz), 7.30–7.44 (5H, m), 7.84 (2H, d, J = 8.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1, 27.9 (3C), 28.2 (3C), 34.3, 38.6, 54.1, 55.4, 56.3, 69.4, 69.9, 71.5, 80.4, 83.1, 113.8 (2C), 114.9 (2C), 125.9, 127.5 (2C), 127.9, 128.6 (2C), 129.2 (2C), 130.2 (2C), 136.9, 157.6, 162.5, 167.2, 169.3, 169.6 and 170.9; HRMS (FAB) $[M+H]^+$: calcd for $C_{41}H_{54}N_3O_{11}$ 764.3758, found 763.3755.; IR (ATR); 2979, 1733, 1652, 1507, 1367, 1247, 1155, 1028 and 751 cm^{-1}; $[\alpha]_D^{19}$ +36.6 (*c* 3.0, $CHCl_3$).

***N*-((2*R*,3*R*,9*S*,10*R*)-9-(4-(Benzyloxy)benzyl)-10-hydroxy-2-methyl-4,7,12-trioxo-1-oxa-5,8-diazacyclododecan-3-yl)-4-methoxybenzamide (21).** ^1H NMR (400 MHz, DMSO-d_6) δ 1.16 (3H, d, J = 6.3 Hz), 2.35–2.48 (2H, m), 2.63 (1H, dd, J = 13.7, 8.6 Hz), 2.94 (1H, dd, J = 13.7, 3.2 Hz), 3.25 (1H, dd, J = 13.2, 3.4 Hz), 3.72 (1H, qd, J = 9.2, 3.4 Hz), 3.80 (3H, s), 3.81–4.05 (2H, m), 4.83 (1H, dd, J = 9.3, 3.6 Hz), 5.03 (2H, s), 5.33 (1H, d, J = 6.6 Hz), 5.56 (1H, qd, J = 6.4, 3.8 Hz), 6.78 (1H, d, J = 9.8 Hz), 6.88 (2H, d, J = 8.8 Hz), 7.00 (2H, d, J = 9.0 Hz), 7.08 (2H, d, J = 8.8 Hz), 7.29–7.44 (5H, m), 7.89 (2H, d, J = 9.0 Hz) and 8.18–8.23 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.9, 36.1, 38.6, 40.9, 44.1, 55.4, 56.2, 56.4, 69.1, 69.4, 70.2, 113.4 (2C), 114.2 (2C), 126.0, 127.6 (2C), 127.7, 128.3 (2C), 129.6 (2C), 130.3 (2C), 130.7, 137.2, 156.6, 161.8, 166.6, 169.0, 169.3 and 170.8; HRMS (FAB) $[M+H]^+$: calcd for $C_{32}H_{36}N_3O_8$ 590.2502, found 590.2468; IR (ATR); 3314, 2926, 1720, 1641, 1606, 1509, 1455, 1254, 1176 and 1021 cm^{-1}; $[\alpha]_D^{21}$ +53.3 (*c* 0.25, $CHCl_3$–MeOH = 1/1).

3-*epi*-Melleumin A (6). ^1H NMR (500 MHz, DMSO-d_6) δ 1.15 (d, J = 6.5 Hz, 3H, H-12), 2.36 (dd, J = 9.8, 15.1 Hz, 1H, H-2), 2.45 (d, J = 15.1 Hz, 1H, H-2), 2.57 (dd, J = 8.5, 13.6 Hz, 1H, H-1″), 2.87 (dd, J = 3.2, 13.6 Hz, 1H, H-1″), 3.25 (dd, J = 4.0, 13.7 Hz, 1H, H-7), 3.67 (m, 1H, H-4), 3.80 (s, 3H, OC*H*$_3$), 3.82 (m, 2H, H-3, H-7), 4.83 (dd, J = 3.7, 9.2 Hz, 1H, H-10), 5.34 (d, J = 6.4 Hz, 1H, CHO*H*), 5.54 (dq, J = 3.7, 6.5 Hz, 1H, H-11), 6.61 (d, J = 8.4 Hz, 2H, H-4″ and H-6″), 6.75 (d, J = 9.5 Hz, 1H, H-5), 6.94 (d, J = 8.4 Hz, 2H, H-3″ and H-5″), 6.99 (d, J = 8.9 Hz, 2H, H-5′ and H-7′), 7.90 (d, J = 8.9 Hz, 2H, H-4′ and H-8′), 8.25 (m, 2H, H-8, H-1′) and 9.11 (br s, 1H, C_6H_4OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 15.6, 36.0, 40.9, 44.1, 55.4, 56.2, 56.5, 69.3, 70.3, 113.4 (2C), 114.8 (2C), 126.0, 128.5, 129.7 (2C), 130.2 (2C), 155.4, 161.8, 166.6, 169.0, 169.3 and 170.9; HRMS (FAB) $[M+Na]^+$: calcd for $C_{25}H_{29}N_3O_8Na$ 522.1852, found 522.1849; IR (ATR); 3291, 2923, 2851, 1732, 1717, 1637, 1516, 1506, 1256, 1177 and 1055 cm^{-1}; $[\alpha]_D^{25}$ +42.3 (*c* 1.0, EtOH).

(3*R*,4*S*)-Ethyl-3-(allyloxy)-5-(4-(benzyloxy)phenyl)-4-(*tert*-butoxycarbonylamino)-pentanoate (22). To a solution of **15** (254 mg, 0.574 mmol), $Pd_2(dba)_3$ (13 mg, 14 mmol) and dppb (25 mg, 0.57 mmol) in THF (2.3 mL) was added allyl ethyl carbonate (0.45 mL, 3.44 mmol) under argon atmosphere. The mixture was heated to 65 °C and stirred for 4 h and then concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–diethyl ether = 5/1 to 1/1) to afford **22** (258 mg, 96%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 1.26 (t, 3H, J = 7.1 Hz), 1.46 (s, 9H), 2.53 (dd, 1H, J = 5.5, 15.5,Hz), 2.57 (dd, 1H, J = 6.5, 15.5 Hz), 2.59–2.69 (m, 1H), 2.91 (dd, 1H, J = 4.4, 14.2 Hz), 3.85–3.92 (m, 2H), 4.02 (ddt, 1H, J = 12.4, 6.0, 1.4 Hz), 4.07–4.18 (3H, m), 4.41 (1H, br s), 5.03 (2H, s), 5.15 (1H, dq, J = 10.3, 1.5 Hz), 5.26 (1H, dq, J = 17.2, 1.7 Hz), 5.85–5.94 (1H, m), 6.88 (2H, d, J = 8.8 Hz), 7.09 (2H, d, J = 8.8 Hz), 7.29–7.42 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 28.0, 35.6, 37.0, 54.6, 60.1, 69.8, 71.0, 77.6, 78.7, 114.7, 116.3, 127.0, 127.4, 128.1, 129.9, 130.3, 134.6, 137.1, 155.0, 157.3 and 171.1; HRMS (EI) $[M]^+$: calcd for $C_{28}H_{37}NO_6$ 483.2621, found 483.2618; IR (ATR); 3345, 2989, 1726, 1683, 1529, 1509, 1237, 1158 and 1004 cm^{-1}; $[\alpha]_D^{19}$ −20.5 (*c* 3.0, $CHCl_3$).

(3*R*,4*S*)-Ethyl-3-allyloxy-4-(2-(9-fluorenylmethoxycarbonylamino)acetamide)-5-(4-(benzyloxy)phenyl)pentanoate (23). To a solution of **22** (242 mg, 0.50 mmol) in CH_2Cl_2 (2.5 mL) was treated with TFA (2.5 mL) at 0 °C under argon atmosphere. After stirring for 1 h at RT, the solvent was removed *in vacuo*. The residue was used without further purification in the following reaction. A solution of Fmoc-glycine (223 mg, 0.750 mmol), PyBOP (390 mg, 0.75 mmol), HOBt·H_2O (101 mg, 0.75 mmol) and DIEA (0.26 mL, 1.50 mmol) in CH_2Cl_2 (2.5 mL) was stirred for 10 min at 0 °C under argon atmosphere. To this mixture was added a solution of the deprotected material obtained above in CH_2Cl_2 (2.5 mL). After stirring overnight, the reaction mixture was quenched with 5% aqueous $KHSO_4$ and extracted with CH_2Cl_2. The organic layer was washed with 5% aqueous $NaHCO_3$, water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–EtOAc = 2/1 to1/1) to afford **23** (321 mg, 0.483 mmol, 61%, 2 steps from **22**) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (t, J = 7.1 Hz, 3H), 2.53 (dd, J = 5.4, 15.6 Hz, 1H), 2.60 (dd, J = 7.1, 15.6 Hz, 1H), 2.68 (dd, J = 9.5, 14.2 Hz, 1H), 2.91 (dd, J = 4.9, 14.2 Hz, 1H), 3.70 (d, J = 5.6 Hz, 2H), 3.86–3.93 (m, 1H), 4.00 (dd, J = 6.1, 13.0 Hz, 1H), 4.07–4.41 (m, 7H), 4.97 (s, 2H), 5.15 (dd, J = 10.3, 1.5 Hz, 1H), 5.25 (dd, J = 17.3, 1.2 Hz, 1H), 5.27 (br s, 1H), 5.82–5.92 (m, 1H), 6.14 (br t, J = 9.0 Hz, 1H), 6.84 (d, J = 8.6 Hz, 2H), 7.04 (d, J = 8.6 Hz, 2H), 7.27–7.42 (m, 9H), 7.58 (br d, J = 7.1 Hz, 2H), and 7.75 (d, J = 7.6 Hz, 2H); ^{13}C NMR 100 MHz ($CDCl_3$) δ 14.1, 35.0, 37.0, 44.4, 47.0, 53.0, 60.8, 67.1, 69.8, 71.3, 114.7, 117.1, 120.0, 125.0, 127.0, 127.4, 127.7, 127.8, 128.4, 129.7, 130.0, 134.4, 136.9, 141.2, 141,6, 156.4, 157.3, 168.4, and 171.4; HRMS (FAB) $[M+Na]^+$: calcd for $C_{40}H_{42}N_2O_7Na$ 685.2890, found 685.2912; IR (ATR); 3307, 3272, 1726, 1696, 1652, 1539, 1509, 1232 and 731 cm^{-1}; $[\alpha]_D^{19}$ −25.2 (*c* 3.0, $CHCl_3$).

(3*R*,4*S*)-Ethyl-3-(allyloxy)-4-(2-((*S*)-2-(*tert*-butoxycarbonylamino)pent-4-enamido)acetamido)-5-(4-(benzyloxy)phenyl)pentanoate (24). A solution of **23** (441 mg, 0.665 mmol) in DMF

(6.7 mL) was treated with piperidine (0.67 mL, 6.7 mmol) for 1 h. The residue after evaporation was used without further purification in the next reaction. A solution of *N*-Boc-L-allylglycine (147 mg, 0.682 mmol), PyBOP (519 mg, 1.00 mmol), $HOBt·H_2O$ (135 mg, 1.00 mmol) and DIPEA (0.35 mL, 1.50 mmol) in DMF (1.5 mL) was stirred for 20 min at 0 °C under argon atmosphere. To this solution was added a solution of the deprotected material obtained above in DMF (3.3 mL). After stirring overnight at RT, the reaction mixture was quenched with 5% aqueous $KHSO_4$ and extracted with dichloromethane. The organic layer was washed with 5% aqueous $NaHCO_3$, water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–EtOAc = 3/1 to 1/2) to afford **24** (352 mg, 0.552 mmol, 83%, 2 steps from **23**) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (t, J = 7.1 Hz, 3H), 1.42 (s, 9H), 2.37–2.60 (m, 4H), 2.67 (dd, J = 9.3, 14.1 Hz, 1H), 2.87 (dd, J = 4.7, 14.1 Hz, 1H), 3.71 (dd, J = 4.6, 16.5 Hz, 1H), 3.86–4.09 (m, 4H), 4.13 (q, J = 7.1 Hz, 2H), 4.15–4.22 (m, 1H), 4.33–4.39 (m, 1H), 4.96 (s, 2H), 5.66–5.91 (m, 2H), 6.86 (d, J = 8.7 Hz, 2H), 7.08 (d, J = 8.7 Hz, 2H) and 7.29–7.40 (m, 5H); ^{13}C NMR 100 MHz ($CDCl_3$) δ 13.9, 28.0 (3C), 35.3, 36.5, 36.6, 42.8, 52.7, 53.8, 60.4, 69.5, 71.0, 76.9, 79.7, 114.5 (2C), 116.7, 118.7, 127.2 (2C), 127.6, 128.2 (2C), 129.8, 130.0 (2C), 132.8, 134.4, 139.8, 155.3, 157.1, 168.2, 171.5, and 171.9; HRMS (FAB) $[M+Na]^+$: calcd for $C_{35}H_{47}N_3O_8Na$ 660.3261, found 660.3248; IR (ATR) 3299, 2979, 1645, 1509, 1366, 1240, 1164, 1020, 920, 737 and 695 cm^{-1}; $[\alpha]_D^{19}$ +4.9 (*c* 3.0, MeOH).

Ethyl-2-((2*R*,3*S*,9*R*,*E*)-3-(4-(benzyloxy)benzyl)-9-(*tert*-butoxycarbonylamino)-5,8-dioxo-1-oxa-4,7-diazacyclotridec-11-en-2-yl)acetate (25). To a solution of **24** (32 mg, 50 µmol) in CH_2Cl_2 (25 mL) was added Grubbs' catalyst (second-generation) (8.4 mg, 10 µmol). The mixture was heated under reflux conditions for 20 min and concentrated *in vacuo*. Purification of this residue by flash chromatography (hexane–EtOAc = 1/1 to 0/1) afforded **25** (30 mg, 98%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 (t, J = 7.1 Hz, 3H), 1.36 (s, 9H), 2.30–2.47 (m, 4H), 2.59 (dd, J = 7.6, 15.8 Hz, 1H), 2.76 (dd, J = 4.4, 14.1 Hz, 1H), 3.29 (dd, J = 4.6, 16.3 Hz, 1H), 3.62–3.76 (m, 3H), 4.00–4.15 (m, 5H), 5.02 (s, 2H), 5.72 (dt, J = 6.6, 15.4 Hz, 1H), 5.80 (dt, J = 6.4, 15.4 Hz, 1H), 6.75 (br d, J = 9.3 Hz, 1H), 6.86 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 7.3 Hz, 1H) and 7.29–7.43 (m, 5H); ^{13}C NMR 100 MHz ($CDCl_3$) δ 14.0, 28.2 (3C), 33.4, 34.6, 37.4, 43.0, 52.0, 53.1, 60.1, 68.7, 69.1, 76.7, 78.2, 114.3 (2C), 127.7 (2C), 127.8, 128.4 (2C), 129.5, 130.1 (2C), 131.0, 131.1, 137.2, 154.9, 156.7, 167.5, 170.7, and 171.5; HRMS (FAB) $[M+Na]^+$: calcd for $C_{33}H_{43}N_3O_8Na$ 632.2948, found 632.2924; IR (ATR) 3325, 2926, 1732, 1655, 1514, 1245, 1170, 1018, 733 and 695 cm^{-1}; $[\alpha]_D^{19}$ –40 (*c* 0.1, $CHCl_3$).

Ethyl-2-((2*R*,3*S*,9*R*,*E*)-3-(4-(benzyloxy)benzyl)-9-(4-methoxybenzamido)-5,8-dioxo-1-oxa-4,7-diazacyclotridec-11-en-2-yl)acetate (26). A solution of **25** (13 mg, 21.6 mmol) in CH_2Cl_2 (1.0 mL) was treated with TFA (1.0 mL) at 0 °C under argon atmosphere. After stirring for 30 min, the solvent was removed *in vacuo*. The residue after evaporation was used without further purification in the next reaction. To a solution of the deprotected material and triethylamine (8 µL, 108 mmol) in CH_2Cl_2 (2.2 mL) was added *p*-methoxybenzoyl chloride (6 µL, 43 mmol) at 0 °C. After stirring overnight at RT, the reaction mixture was quenched with 5% aqueous $NaHCO_3$, and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by preparative TLC (silica gel, MeOH–$CHCl_3$ = 1/10), to give **26** (8 mg, 54%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$–CD_3OD = 4/1) δ 1.24 (t, J = 7.0 Hz, 3H), 2.37 (dd, J = 5.4, 15.6 Hz, 1H), 2.54 (dd, J = 7.6, 15.6 Hz, 1H), 2.61–2.69 (m, 2H), 2.78–2.85 (m, 1H), 2.88 (dd, J = 5.9, 14.0 Hz, 1H), 3.81 (s, 3H), 3.87 (dd, J = 6.2, 13.1 Hz, 1H), 3.92 (ddd, J = 1.5, 5.4, 7.6 Hz, 1H), 4.02 (dd, J = 6.3, 13.5 Hz, 1H), 4.12 (q, J = 7.0 Hz, 2H), 4.17 (dd, J = 6.2, 13.1 Hz, 1H), 4.20–4.26 (m, 1H), 4.81–4.86 (m, 1H), 5.01 (s, 2H), 5.85 (dt, J = 6.3, 15.6 Hz, 1H), 5.92 (dt, J = 6.2, 15.6 Hz, 1H), 6.63 (br d, J = 9.2 Hz, 1H), 6.87 (d, J = 8.6 Hz, 2H), 6.95 (d, J = 8.7 Hz, 2H), 6.95 (d, J = 7.3 Hz, 2H), 7.14 (d, J = 8.5 Hz, 2H), 7.29–7.43 (m, 5H), and 7.72 (d, J = 8.7 Hz, 2H); ^{13}C NMR 125 MHz ($CDCl_3$) δ 14.2, 34.2, 35.5, 38.4, 43.8, 52.8, 53.0, 55.4, 60.8, 69.8, 69.9, 77.6, 113.9 (2C), 114.6 (2C), 125.3, 127.5 (2C), 127.9, 128.5 (2C), 129.0 (2C), 130.2, 130.4 (2C), 130.5, 131.2, 137.1, 157.3, 162.7, 167.0, 167.3, 171.0, and 171.6; HRMS (FAB) $[M+H]^+$: calcd for $C_{33}H_{38}N_3O_7$ 644.2941, found 644.2971; IR (ATR) 3281, 2923, 1746, 1661, 1540, 1507,1253, 1177, 1025 and 695 cm^{-1}; $[\alpha]_D^{24}$ –22 (*c* 0.2, DMSO).

Ethyl-2-((2*R*,3*S*,9*R*,*E*)-3-(4-(benzyloxy)benzyl)-5,8-dioxo-9-(2,4,6-trichlorobenzamido)-1-oxa-4,7-diazacyclotridec-11-en-2-yl)acetate (27). ^{1}H NMR (500 MHz, $CDCl_3$–CD_3OD = 4/1) δ 1.24 (t, J = 7.0 Hz, 3H), 2.37 (dd, J = 5.4, 15.6 Hz, 1H), 2.54 (dd, J = 7.6, 15.6 Hz, 1H), 2.61–2.69 (m, 2H), 2.78–2.85 (m, 1H), 2.88 (dd, J = 5.9, 14.0 Hz, 1H), 3.81 (s, 3H), 3.87 (dd, J = 6.2, 13.1 Hz, 1H), 3.92 (ddd, J = 1.5, 5.4, 7.6 Hz, 1H), 4.02 (dd, J = 6.3, 13.5 Hz, 1H), 4.12 (q, J = 7.0 Hz, 2H), 4.17 (dd, J = 6.2, 13.1 Hz, 1H), 4.20–4.26 (m, 1H), 4.81–4.86 (m, 1H), 5.01 (s, 2H), 5.85 (dt, J = 6.3, 15.6 Hz, 1H), 5.92 (dt, J = 6.2, 15.6 Hz, 1H), 6.63 (br d, J = 9.2 Hz, 1H), 6.87 (d, J = 8.6 Hz, 2H), 6.95 (d, J = 8.7 Hz, 2H), 6.95 (d, J = 7.3 Hz, 2H), 7.14 (d, J = 8.5 Hz, 2H), 7.29–7.43 (m, 5H), and 7.72 (d, J = 8.7 Hz, 2H); ^{13}C NMR 125 MHz ($CDCl_3$) δ 14.2, 34.2, 35.5, 38.4, 43.8, 52.8, 53.0, 55.4, 60.8, 69.8, 69.9, 77.6, 113.9 (2C), 114.6 (2C), 125.3, 127.5 (2C), 127.9, 128.5 (2C), 129.0 (2C), 130.2, 130.4 (2C), 130.5, 131.2, 137.1, 157.3, 162.7, 167.0, 167.3, 171.0, and 171.6; HRMS (FAB) $[M+H]^+$: calcd for $C_{35}H_{37}Cl_3N_3O_7$ 716.1697, found 716.1681; IR (ATR) 3281, 2923, 1746, 1661, 1540, 1507,1253, 1177, 1025 and 695 cm^{-1}; $[\alpha]_D^{24}$ –22 (*c* 0.2, DMSO).

Ethyl-2-((2*R*,3*S*,9*R*)-3-(4-hydroxybenzyl)-9-(4-methoxybenzamido)-5,8-dioxo-1-oxa-4,7-diazacyclotridecan-2-yl)acetate (28). To a solution of **26** (5 mg, 8 µmol) in solvent (THF–EtOH = 2/1) was added 25% palladium on carbon (1 mg). The reaction mixture was stirred for 7 h under hydrogen atomosphere and then filtered through Celite and concentrated *in vacuo*. The crude product was purified by preparative TLC (silica gel, MeOH–$CHCl_3$ = 1/9), to give **28** (3 mg, 6 µmol, 70%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD = 4/1) δ 1.29 (t, J = 7.1 Hz, 3H), 1.49–1.92 (m, 6H), 2.49 (dd, J = 6.6, 15.3 Hz, 1H), 2.62 (dd, J = 10.0, 14.2 Hz, 1H), 2.69 (dd, J = 6.6, 15.3 Hz, 1H), 2.89 (dd, J = 4.9, 14.2 Hz, 1H), 3.23 (t, J = 11.7 Hz, 1H), 3.46 (d, J = 16.5 Hz, 1H), 3.66–3.85 (m, 2H), 3.87 (s, 3H), 3.92 (d, J = 16.5 Hz, 1H), 4.16 (q, J = 7.1 Hz, 2H), 4.21–4.29 (m, 1H), 4.47 (dd, J = 3.1, 9.9 Hz, 1H), 6.73 (d, J = 8.6 Hz, 2H), 6.94 (d, J = 8.9 Hz, 2H), 7.04 (d, J = 8.6 Hz, 2H), and 7.79 (d, J = 8.9 Hz, 2H); ^{13}C NMR 125 MHz ($CDCl_3$) δ 14.2, 20.9, 28.5, 31.8, 33.6, 37.2, 43.5, 53.0, 54.2, 55.6,

61.3, 68.1, 78.7, 114.0 (2C), 115.3 (2C), 125.8, 129.0 (2C), 129.3, 130.4 (2C), 155.5, 162.8, 167.5, 168.6, 171.5 and 173.7; HRMS (FAB) $[M+H]^+$: calcd for $C_{29}H_{38}N_3O_8$ 556.2659, found 556.2608; IR (ATR) 3394, 3290, 2920, 1731, 1651, 1540, 1506,1458, 1258, 1183, 1110, 1026 and 846 cm^{-1}; $[\alpha]_D^{19}$ −16.9 (*c* 0.5, MeOH).

Cell cultures. STF/293 cells were a generous gift from Prof. Jeremy Nathans (Johns Hopkins Medical School). STF/293 and 293T cells were cultured in Dulbecco's modified eagle medium (Wako) with 10% FBS. Cultures were maintained in a humidified incubator at 37 °C in 5% CO_2/95% air.

Super TOP-Flash reporter assay. STF/293 cells (a 293 human embryonic kidney cell line stably transfected with Super Top-Flash, 3×10^4) were split into 96-well plates and 24 h later cells were treated with 15 mM LiCl and testing samples (DMSO solution) with a medium containing FBS. After incubation for 24 h, cells were lysed with CCLR (cell culture lysis reagent; 20 μL/well, Promega) and luciferase activities were measured with a Luciferase Assay System (Promega). We checked this system worked correct by using quercetin as a standard positive compound. Assays were performed in triplicate.

Super FOP-Flash reporter assay. 293T cells (1×10^5) were split into 24-well plates and were transfected 24 h later with 1 μg/well Super Fop-Flash reporter plasmid and 0.05 μg of pRL-CMV plasmid (Promega, USA) for normalization using Lipofectamine2000 (Invitrogen; 2.5 μL/well). After 3 h transfection, compounds were added with a medium containing FBS. Of note, 293T cells were treated with compounds in a FBS-containing medium combined with 15 mM of LiCl. Cells incubated for 24 h were lysed in Passive lysis buffer (Promega, 50 μL/well) and luciferase activity was measured with a Dual-Glo Luciferase Assay System (Promega).

Assay of cell viability. 293T cells (6×10^3) were split into 96-well plates and incubated for 24 h. Cells were treated with compounds and incubated for 24 h. They were treated with fluorescein diacetate (Wako) in PBS buffer (10 μg mL^{-1}), and after 1 h of incubation, fluorescence was detected. Assays were performed at least in triplicate.

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