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SYNTHESIS OF SUNITINIB–METASTIN CONJUGATE, A NOVEL ESTERASE-SENSITIVE PRODRUG SYSTEM BASED ON LACTONIZATION REACTION

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Abstract – We describe a strategy for preparing sunitinib–metastin conjugate, a prodrug composed of the anticancer agent sunitinib for renal cell carcinoma and the carrier protein metastin, which are conjugated to each other by a linker. We designed a modified L-homoserine linker, which is composed of an acyl group that acts as the masking group for hydrolysis with an esterase, as well as a carbon chain of appropriate length between sunitinib and metastin. The sunitinib–metastin conjugate was converted into a hydrolyte by hydrolysis of the acyl group with an esterase, and sunitinib was released by intramolecular lactonization. Sunitinib–metastin conjugate, an esterase-sensitive amide prodrug that has a modified L-homoserine linker that participates in the intramolecular lactonization, was synthesized.

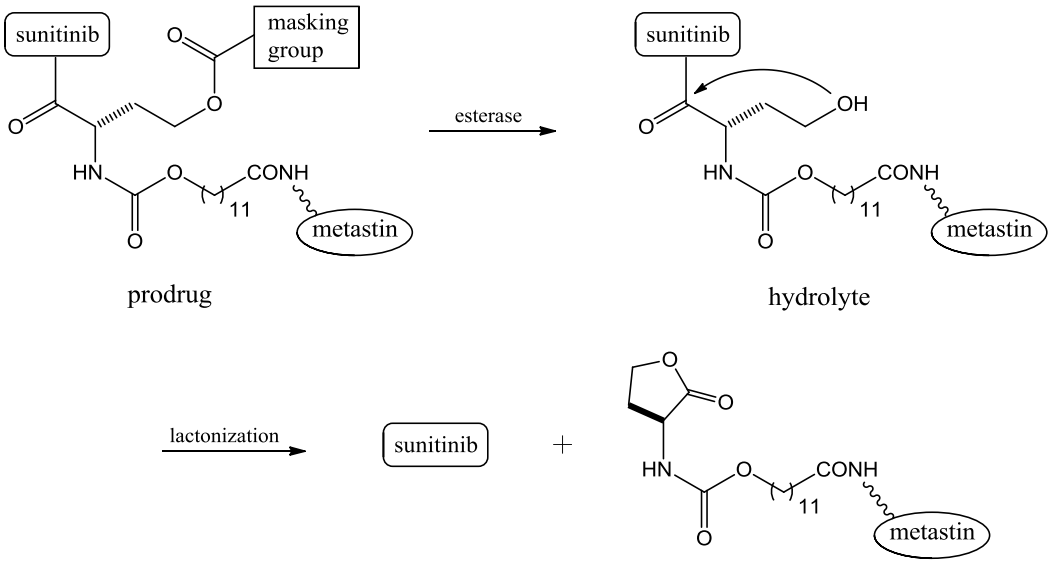
INTRODUCTION

The *KISS-1* gene is located on human chromosome lq32.[1,2] *KISS-1* encodes a C-terminus peptide with 54 amino acid residues called metastin (kisspeptin), which is the ligand of the G-protein-coupled metastin

receptor.[3] Both metastin and the metastin receptor inhibit tumor invasion or migration through focal adhesion kinase, paxillin, MAP kinase or Rho A, and have been implicated in melanoma, thyroid cancer, esophageal squamous cell carcinoma, hepatocellular carcinoma, pancreatic carcinoma, breast cancer, ovarian cancer, renal cell carcinoma (RCC), upper tract urothelial carcinoma, bladder cancer, and prostate cancer.[4-6] Metastin and the metastin receptor are expressed in the hypothalamus, brainstem, spinal cord, pituitary, ovary, prostate, and placenta in normal human tissue, and play a pivotal role in the control of the hypothalamic-pituitary-gonadal axis via regulation of gonadotropin-releasing hormone secretion.[7] Moreover, it is reported that the metastin receptor is overexpressed in human RCC, and that metastin and the metastin receptor are probable targets for suppressing RCC metastasis.[8]

Drug delivery systems (DDSs), which are engineered technologies for the targeted delivery and controlled release of a therapeutic agent, have been the focus of intensive studies. The delivery of an anticancer agent directly and locally to an affected part of the body is expected to yield high curative effects without producing any side effects. Generally, anticancer agents show low selectivity for tumor sites, the development of anticancer agents having a protein or a monoclonal antibody that shows affinity for tumor sites, and the control of their pharmacokinetics *in vivo* have been carried out. As the interaction between a ligand and a receptor has high specificity, and the development of a prodrug that uses metastin as the carrier protein would be a valuable contribution to the treatment of RCC. Although sunitinib is the active drug for RCC treatment as tyrosine kinase inhibitor, there are many systemic side effects. As far as we know, there is no report of a prodrug that uses metastin as the carrier protein and sunitinib. In order to reduce the risk of systemic side effects of sunitinib, we decided to synthesize sunitinib–metastin conjugate with metastin as the carrier protein. To this end, we embarked on the synthesis of the sunitinib–metastin conjugate and examined its potential for use as a prodrug for RCC treatment.

The design concept of the linker between sunitinib and metastin was considered as follows. Sunitinib should be released upon lactonization of the hydrolyte produced by the esterase hydrolysis of the sunitinib–metastin conjugate (Scheme 1).[9] Therefore, the linker should have an acyl group as the masking group, which should be removed by esterase hydrolysis *in vivo*, and should form a γ-lactone by intramolecular lactonization. Moreover, if the linker has a short carbon chain between sunitinib and metastin, the proximity of metastin to sunitinib would inhibit the binding of metastin to the metastin receptor. Accordingly, we decided to conjugate metastin to the linker terminus with an extended carbon chain. Finally, we designed a modified L-homoserine linker, which is composed of an acyl group that acts as the masking group for hydrolysis with an esterase, as well as a carbon chain of appropriate length between sunitinib and metastin.



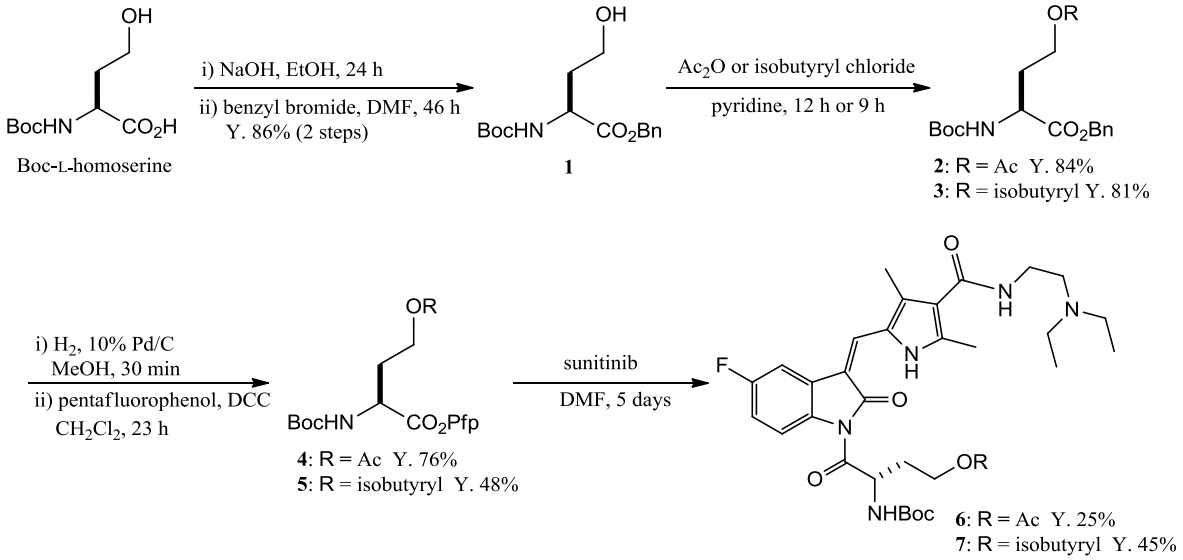
Scheme 1. Sunitinib releasing model through lactonization of the L-homoserine linker

We initially investigated the binding position of sunitinib to the modified L-homoserine linker and the optimization of the acyl group as the masking group. Because the structure of sunitinib made it difficult to bind to the linker, we decided to connect the linker to the nitrogen atom of the oxyindole ring of sunitinib. Furthermore, the linker terminus, which was connected to metastin, had to be a carboxylic acid. Therefore, the linker was synthesized by using an acetyl or an isobutyryl group as the acyl group, and then connected to sunitinib. Compounds having an acetyl or an isobutyryl group as the masking group were evaluated for the release of sunitinib by enzyme hydrolysis under physiological conditions *in vitro*. An aliquot of the reaction mixture was subjected to HPLC to monitor the reaction. The synthesis of the amide prodrug, sunitinib–metastin conjugate, which has a modified L-homoserine linker, is described in detail below.

RESULTS AND DISCUSSION

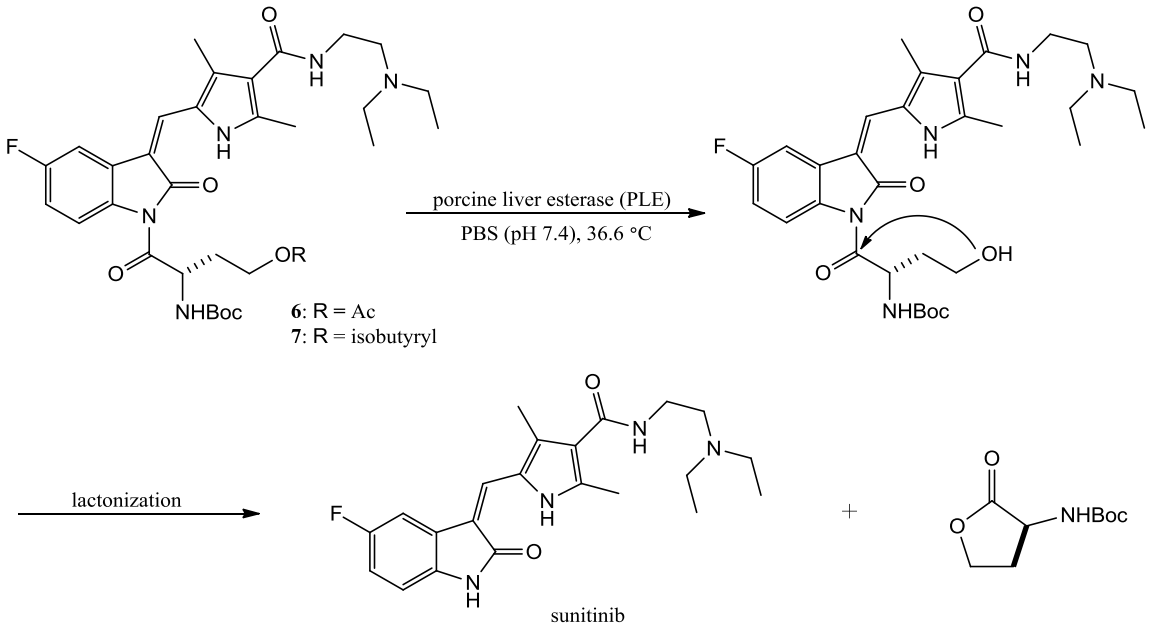
The linker having an acetyl group or an isobutyryl group as the masking group was synthesized by the following procedure (Scheme 2). Compounds **6** and **7** were synthesized from commercially available Boc-L-homoserine. Boc-L-homoserine was converted into benzyl ester **1**, which was then treated with acetic anhydride in pyridine to provide acetate **2**. The benzyl group of **2** was removed with hydrogen gas and 10% Pd/C, and the resultant carboxylic acid was treated with pentafluorophenol in the presence of *N*,*N*'-dicyclohexylcarbodiimide (DCC) in CH_2Cl_2 to give activated ester **4**. Treatment of **4** with sunitinib in *N*,*N*-dimethylformamide (DMF) afforded compound **6**. Compound **7**, which possesses an isobutyryl

group as the masking group, was synthesized in a similar way from compound **1** via compound **3** using isobutyryl chloride instead of acetic anhydride.



Scheme 2

Compounds **6** and **7** were evaluated to determine whether sunitinib is released by the intramolecular lactonization of the hydrolyte obtained, by hydrolysis with porcine liver esterase (PLE) (Scheme 3).



Scheme 3

First, compound **6** was converted into the malic acid salt, and this was dissolved in phosphate-buffered saline (PBS, pH 7.4) and left to stand at 36.6 °C with or without PLE. The reaction was monitored by HPLC equipped with a photodiode array detector to measure the absorption spectrum of sunitinib at the maximum absorption wavelength of 431 nm (Figures 1 and 2).[10]

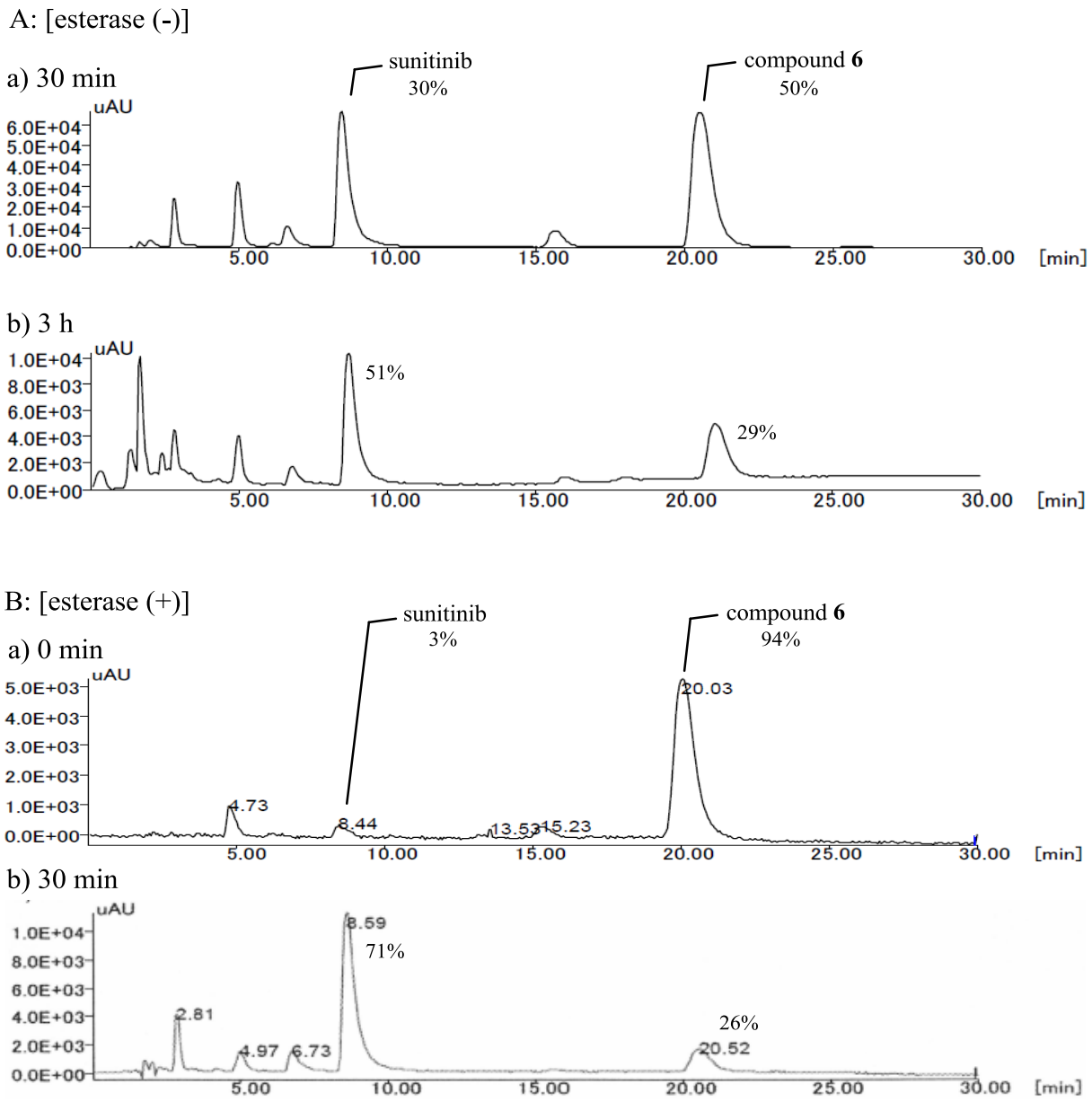


Figure 1. HPLC chromatographic profiles of compound **6** (A: without esterase as a blank, B: with esterase)
HPLC conditions: column, NUCLEODUR 3CN column (3 μm, 4.6 x 150 mm, Chemco Inc.); mobile phase, MeCN/aqueous NH_4OAc (20 mmol/L, pH 6.8)=55:45; flow rate, 1 mL/min; temperature, 40 °C; wavelength, 431 nm.

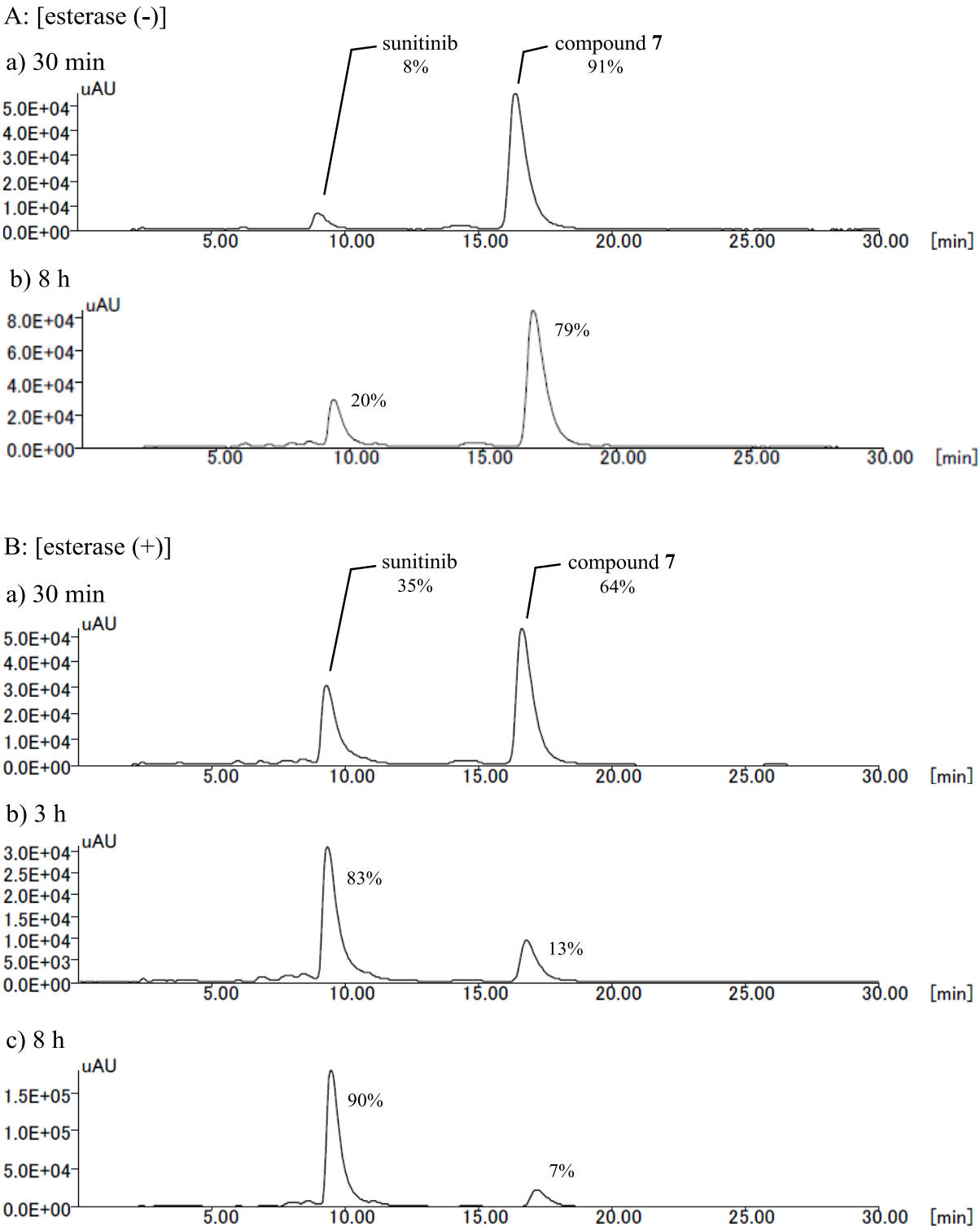
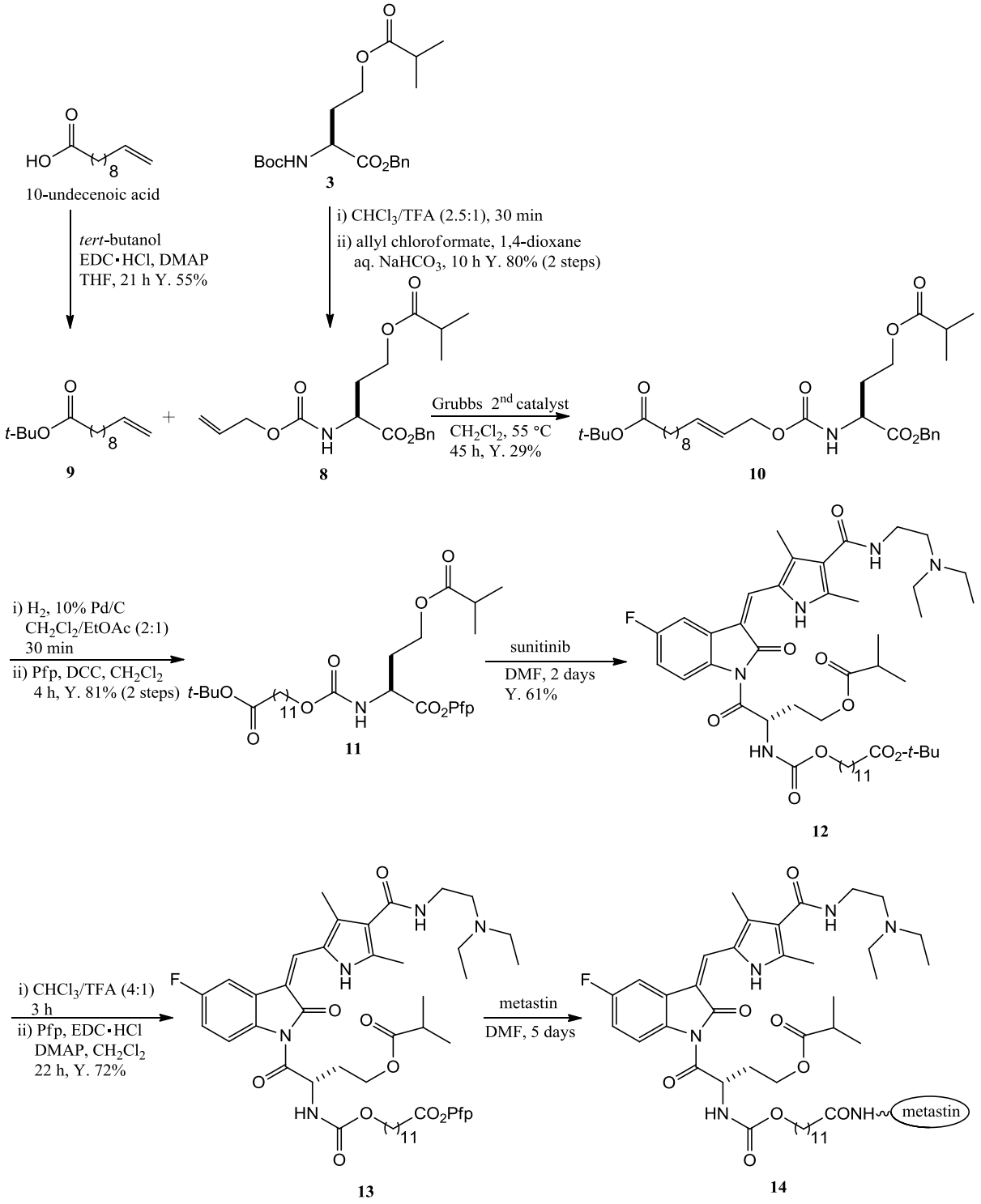


Figure 2. HPLC chromatographic profiles of compound **7** (A: without esterase as a blank, B: with esterase)
For HPLC conditions, see Figure 1.

The t_R values for sunitinib, compound **6**, and compound **7** were 8, 20, and 16 min, respectively. It was observed that compound **6** having an acetyl group released sunitinib via the hydrolyte in the absence of

PLE at 3 h after the start of the reaction (Figure 1A). In the case of compound **7** having an isobutyryl group, sunitinib was released slightly in the absence of PLE at 8 h after the start of the reaction, and was released in the presence of PLE at 3 h after the start of the reaction (Figure 2). The results indicated that the isobutyryl group was a more suitable masking group than the acetyl group.



Scheme 4

Sunitinib–metastin conjugate **14** was synthesized by the following procedure (Scheme 4).

10-Undecenoic acid was treated with *tert*-butanol, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) in THF to provide compound **9**. The Boc group of compound **3** was removed by treating **3** with $CHCl_3$/trifluoroacetic acid (TFA) (2.5:1), and the product was reacted with allyl chloroformate in 1,4-dioxane/saturated aqueous $NaHCO_3$ (3:1) to afford compound **8**. Compound **8** was reacted with compound **9** in the presence of 0.2 molar equiv of Grubbs 2nd generation catalyst in CH_2Cl_2 to yield compound **10**. The benzyl group of compound **10** was removed by treating **10** under an atmosphere of hydrogen with 10% Pd/C, and the product was then treated with pentafluorophenol and DCC in CH_2Cl_2 to provide compound **11**. Compound **11** was mixed with sunitinib in DMF at room temperature for two days to obtain compound **12**. The *t*-butyl group of compound **12** was removed by treating **12** with $CHCl_3$/TFA (4:1), and the product was then treated with pentafluorophenol, EDC·HCl, and DMAP in CH_2Cl_2 to provide compound **13**. Compound **13** was mixed with metastin in DMF at room temperature for five days to furnish sunitinib–metastin conjugate **14**.

The properties of conjugate **14** were determined by ESI-TOF-MS analysis. Figure 3 shows the ESI-TOF-MS spectrum of conjugate **14**.

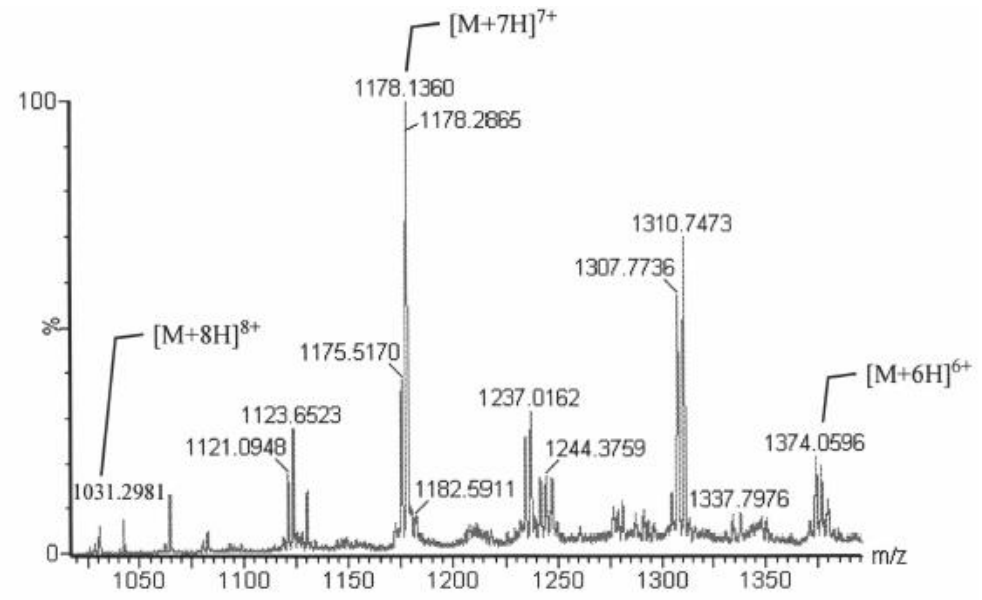


Figure 3. ESI-TOF-MS spectrum of compound **14**

Table. Theoretical average *m*/*z* values for charge states of conjugate **14**

	conjugate **14** (*m*/*z* value)			
	sunitinib-linker (mol)/metastin (1 mol)			
charge	1	2	3	4
1	6650	7445	8239	9031
2	3325	3722	4119	4515
3	2217	2481	2746	3010
4	1663	1861	2059	2258
5	1330	1489	1647	1806
6	1108	1241	1373	1505
7	950	1063	1177	1290
8	831	930	1030	1129
9	739	827	915	1003
10	665	744	824	904

The theoretical average *m*/*z* values for the individual charge states are given in the table. The ESI-TOF-MS spectrum of conjugate **14** showed multiply charged ions at *m*/*z* 1031.2981, 1178.1360, and 1374.0596. When the conjugate has three sunitinib-linker molecules per unit molecule of metastin, the values for *m*/*z* 1031.2981, 1178.1360, and 1374.0596 were $[M+8H]^{8+}$, $[M+7H]^{7+}$, and $[M+6H]^{6+}$, respectively. The results suggest that conjugate **14** is composed of about three sunitinib-linker molecules per unit molecule of metastin.

EXPERIMENTAL

General Experimental Procedures

Thin layer chromatography (TLC) was performed on Merck precoated silica gel 60 F_{254} plates. Optical rotations were measured on a JASCO P-1030 digital polarimeter. IR spectra were recorded on a JASCO FT/IR 620 spectrophotometer, and NMR spectra, on a Bruker AV-600 spectrometer (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR). ^{1}H chemical shifts in $CDCl_3$ were referenced to residual $CHCl_3$ (7.26 ppm), and ^{13}C chemical shifts, to $CDCl_3$ (77.03 ppm). Mass spectra were recorded on a Waters Micromass LCT spectrometer. Analytical HPLC was carried out on a PU-980 pump unit (JASCO) equipped with a multiwavelength detector MD-910, and a NUCLEODUR 3CN column (3 μm, 4.6 × 150 mm, Chemco Inc.). Preparative HPLC was carried out on a PU-986 pump unit (JASCO) equipped with a UV-970 detector (254 nm), and a CAPCELL PAK TYPE UG80 column (5 μm, 20 × 250 mm, Shiseido Co., Ltd.).

(*S*)-Benzyl 2-((*tert*-butoxycarbonyl)amino)-4-hydroxybutanoate (1): Boc-L-homoserine (300 mg, 1.3 mmol) was dissolved in EtOH (2.0 mL) to prepare a solution, to which 2.0 mol/L aqueous NaOH (690 μL,

1.4 mmol) was added. The mixture was stirred at room temperature for 24 h. The solvent was removed *in vacuo*, and the residue was dissolved in DMF (400 μL) together with benzyl bromide (327 μL, 2.7 mmol). The mixture was stirred at room temperature for 46 h. The solvent was removed *in vacuo* and the residue was dissolved in $CHCl_3$ (10 mL). Saturated aqueous $NaHCO_3$ (10 mL) was added to the solution, and the whole was extracted with $CHCl_3$ (3 × 10 mL). The combined $CHCl_3$ extracts were washed with brine (10 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. The residue was subjected to column chromatography (silica gel, 2:1 hexane/EtOAc) to afford **1** (345 mg, 86%) as a colorless amorphous gum: $[\alpha]^{25}_D$ -38.5 (c 0.21, MeOH); IR (film) ν 3356, 1777, 1685, 1532, 1163 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 7.39–7.36 (4H, m), 7.32–7.29 (1H, m), 5.10 (1H, br s), 4.71 (2H, s), 4.45 (1H, t, *J* = 8.8 Hz), 4.36 (1H, br s), 4.25 (1H, ddd, *J* = 11.3, 9.4, 5.8 Hz), 2.67–2.62 (1H, m), 2.11–2.06 (1H, m), 1.47 (9H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.3 (s), 155.5 (s), 140.9 (s), 128.6 × 2 (d), 127.7 (d), 127.0 × 2 (d), 80.7 (s), 65.8 (t), 65.4 (t), 50.2 (d), 30.7 (t), 28.3 × 3 (q); HR-ESIMS *m/z* calcd for $C_{16}H_{24}NO_5$ $[M+H]^+$ 310.1654, found 310.1635.

(*S*)-Benzyl 4-acetoxy-2-((*tert*-butoxycarbonyl)amino)butanoate (2): A solution of **1** (23.8 mg, 0.077 mmol) in pyridine (2.0 mL) was treated with acetic anhydride (1.0 mL). The mixture was stirred at room temperature for 12 h. The solvent was removed *in vacuo* and the residue was dissolved in $CHCl_3$ (10 mL). Saturated aqueous $NaHCO_3$ (10 mL) was added to the solution, and the whole was extracted with $CHCl_3$ (3 × 10 mL). The combined $CHCl_3$ extracts were washed with brine (10 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. The residue was subjected to column chromatography (silica gel, 3:1 hexane/EtOAc) to afford **2** (22.7 mg, 84%) as a colorless amorphous gum: ^1H NMR (600 MHz, $CDCl_3$) δ 7.42–7.33 (5H, m), 5.19 (2H, s), 5.15 (1H, d, *J* = 11.8 Hz), 4.48–4.44 (1H, m), 4.19 (1H, dq, *J* = 12.3, 6.8 Hz), 4.12 (1H, dq, *J* = 12.3, 6.8 Hz), 2.23–2.17 (1H, m), 2.09–2.02 (1H, m), 2.01 (3H, s), 1.45 (9H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.1 (s), 170.8 (s), 155.3 (s), 135.3 (s), 128.7 × 2 (d), 128.5 (d), 128.3 × 2 (d), 80.1 (s), 67.3 (t), 60.4 (t), 51.0 (d), 31.3 (t), 28.3 × 3 (q), 20.8 (q); HR-ESIMS *m/z* calcd for $C_{18}H_{26}NO_6$ $[M+H]^+$ 352.1760, found 352.1763.

(*S*)-Benzyl 2-((*tert*-butoxycarbonyl)amino)-4-(isobutyryloxy)butanoate (3): To a solution of **1** (413 mg, 1.34 mmol) in pyridine (400 μL) was added isobutyryl chloride (141 μL, 1.34 mmol), and the mixture was stirred at room temperature for 9 h. The solvent was removed *in vacuo* and the residue was dissolved in $CHCl_3$ (10 mL). Saturated aqueous $NaHCO_3$ (20 mL) was added to the solution, and the whole was extracted with $CHCl_3$ (3 × 10 mL). The combined $CHCl_3$ extracts were washed with brine (20 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. The residue was subjected to column chromatography (silica gel, 3:1 hexane/EtOAc) to afford **3** (413 mg, 81%) as a colorless amorphous gum: $[\alpha]^{25}_D$ -26.6 (c 0.26, MeOH); IR (film) ν 3369, 2975, 1715, 1516, 1366, 1159 cm^{-1}. ^1H

NMR (600 MHz, $CDCl_3$) δ 7.40–7.35 (5H, m), 5.19 (2H, s), 5.16–5.13 (1H, m), 4.48 (1H, q, *J* = 6.2 Hz), 4.19–4.14 (1H, m), 4.11 (1H, dd, *J* = 12.4, 5.3 Hz), 2.51 (1H, quint, *J* = 6.8 Hz), 2.22–2.17 (1H, m), 2.06–1.99 (1H, m), 1.45 (9H, s), 1.16 (6H, d, *J* = 6.8 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.9 (s), 172.1 (s), 155.2 (s), 135.2 (s), 128.6 × 2 (d), 128.5 (d), 128.3 × 2 (d), 80.1 (s), 67.3 (t), 60.4 (t), 51.1 (d), 33.9 (d), 31.2 (t), 28.3 × 3 (q), 18.9 × 2 (q); HR-ESIMS *m/z* calcd for $C_{20}H_{30}NO_6$ $[M+H]^+$ 380.2073, found 380.2074.

(*S*)-Pentafluorophenyl 4-acetoxy-2-((*tert*-butoxycarbonyl)amino)butanoate (4): 10% Pd/C (44.0 mg) was added to a solution of **2** (44.0 mg, 0.13 mmol) in MeOH (2.0 mL), and the mixture was stirred at room temperature under an atmosphere of hydrogen for 30 min. The catalyst was filtered off, and the filtrate was concentrated to dryness. The residue was dissolved in CH_2Cl_2 (500 μL) together with pentafluorophenol (116 mg, 0.63 mmol) and DCC (36.4 mg, 0.18 mmol). The mixture was stirred at room temperature for 23 h. Saturated aqueous $NaHCO_3$ (30 mL) was added, and the whole was extracted with $CHCl_3$ (3 × 10 mL). The combined $CHCl_3$ extracts were washed with brine (30 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. The residue was subjected to column chromatography (silica gel, 4:1 hexane/EtOAc) to afford **4** (42.3 mg, 76%) as a colorless amorphous gum: ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.19 (1H, d, *J* = 7.3 Hz), 4.78–4.77 (1H, m), 4.31–4.26 (1H, m), 4.21 (1H, dq, *J* = 12.3, 7.3 Hz), 2.41–2.33 (1H, m), 2.24–2.16 (1H, m), 2.01 (3H, s), 1.46 (9H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8 (s), 168.7 (s), 155.1 (s), 141.8 (d), 140.6 (d), 140.2 (d), 138.8 (d), 137.1 (d), 124.7 (d), 80.8 (s), 60.0 (t), 50.9 (d), 30.9 (t), 28.2 × 3 (q), 20.8 (q); HR-ESIMS *m/z* calcd for $C_{17}H_{18}F_5NO_6Na$ $[M+Na]^+$ 450.0952, found 450.0942.

(*S*)-Pentafluorophenyl 2-((*tert*-butoxycarbonyl)amino)-4-(isobutyryloxy)butanoate (5): 10% Pd/C (102 mg) was added to a solution of **3** (151 mg, 0.40 mmol) in MeOH (6.1 mL), and the mixture was stirred at room temperature under an atmosphere of hydrogen for 30 min. The catalyst was filtered off, and the filtrate was concentrated to dryness. The residue was dissolved in CH_2Cl_2 (1.5 mL) together with pentafluorophenol (355 mg, 1.93 mmol) and DCC (172 mg, 0.83 mmol). The mixture was stirred at room temperature for 20 h. Saturated aqueous $NaHCO_3$ (30 mL) was added, and the whole was extracted with $CHCl_3$ (3 × 10 mL). The combined $CHCl_3$ extracts were washed with brine (30 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. The residue was subjected to column chromatography (silica gel, 4:1 hexane/EtOAc) and then HPLC (60:40 MeCN/H_2O) to afford **5** (87.7 mg, 48%) as a colorless amorphous gum: $[\alpha]^{25}_D$ -20.3 (c 0.55, $CHCl_3$); IR (film) ν 3359, 2978, 1794, 1718, 1521, 1157 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.21 (1H, br d, *J* = 8.0 Hz), 4.77 (1H, dd, *J* = 11.5, 6.4 Hz), 4.28 (1H, quint, *J* = 5.6 Hz), 4.22–4.19 (1H, m), 2.56 (1H, sept, *J* = 7.0 Hz), 2.38–2.36 (1H, m), 2.20–2.17 (1H, m), 1.45 (9H, s), 1.176 (3H, d, *J* = 7.0 Hz), 1.175 (3H, d, *J* = 7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.9

(s), 168.6 (s), 155.0 (s), 141.9 (s), 141.8 (s), 140.2 (s), 138.8 (s), 137.1 × 2 (s), 80.8 (s), 60.1 (t), 51.1 (d), 33.9 (d), 30.9 (t), 28.2 × 3 (q), 18.89 (q), 18.86 (q); HR-ESIMS *m/z* calcd for $C_{19}H_{22}F_5NO_6Na$ $[M+Na]^+$ 478.1265, found 478.1257.

(*S*,*Z*)-3-((*tert*-Butoxycarbonyl)amino)-4-(3-((4-((2-(diethylamino)ethyl)carbamoyl)-3,5-dimethyl-1*H*-pyrrol-2-yl)methylene)-5-fluoro-2-oxoindolin-1-yl)-4-oxobutyl acetate (6): Free sunitinib was obtained by the following operations. A solution of sunitinib malate in $CHCl_3$ (30 mL) was treated with aqueous NaOH (1.0 mol/L, 30 mL), washed with brine (30 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. Compound **4** (10.2 mg, 0.024 mmol) and sunitinib (5.4 mg, 0.014 mmol) were dissolved in DMF (200 μL), and the mixture was stirred at room temperature for five days. The solvent was removed *in vacuo*, and the residue was subjected to column chromatography (silica gel, 7:1 $CHCl_3$/MeOH) to afford **6** (2.2 mg, 25%) as an orange amorphous solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.74 (1H, br s), 8.18 (1H, dd, *J* = 8.9, 4.7 Hz), 7.40 (1H, s), 7.19 (1H, dd, *J* = 8.4, 2.6 Hz), 6.92 (1H, td, *J* = 8.9, 2.6 Hz), 6.65 (1H, br s), 5.92 (1H, br s), 5.57 (1H, br d, *J* = 7.6 Hz), 4.28 (2H, dq, *J* = 26.0, 5.9 Hz), 3.54–3.49 (2H, m), 2.73 (2H, br s), 2.63–2.59 (4H, m), 2.53 (3H, s), 2.42 (3H, s), 2.36–2.31 (1H, m), 2.09–1.97 (1H, m), 1.94 (3H, s), 1.56 (9H, s), 1.08 (6H, t, *J* = 7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.3 (s), 170.8 × 2 (s), 168.2 (s), 160.1 (s), 159.6 (s), 155.5 (s), 140.1 (s), 133.8 (d), 128.3 (s), 126.5 (s), 125.2 (s), 120.8 (s), 117.8 (d), 113.2 (d), 111.1 (s), 103.9 (d), 80.2 (s), 60.9 (t), 52.6 (d), 51.4 (t), 46.6 × 2 (t), 36.7 (t), 32.0 (t), 28.4 × 3 (q), 20.9 (q), 14.4 (q), 11.5 × 3 (q); HR-ESIMS *m/z* calcd for $C_{33}H_{45}FN_5O_7$ $[M+H]^+$ 642.3303, found 642.3317.

(*S*,*Z*)-3-((*tert*-Butoxycarbonyl)amino)-4-(3-((4-((2-(diethylamino)ethyl)carbamoyl)-3,5-dimethyl-1*H*-pyrrol-2-yl)methylene)-5-fluoro-2-oxoindolin-1-yl)-4-oxobutyl isobutyrate (7): Compound **5** (14.6 mg, 0.032 mmol) and sunitinib (7.9 mg, 0.020 mmol) were dissolved in DMF (300 μL), and the mixture was stirred at room temperature for five days. The solvent was removed *in vacuo*, and the residue was subjected to column chromatography (silica gel, 7:1 $CHCl_3$/MeOH) to afford **7** (6.0 mg, 45%) as an orange amorphous solid: $[\alpha]^{25}_D$ +42.8 (c 0.23, $CHCl_3$); IR (film) ν 2974, 1698, 1570, 1521, 1474 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.74 (1H, s), 7.93 (1H, br t, *J* = 5.7 Hz), 7.31 (1H, s), 7.15 (1H, dd, *J* = 8.7, 2.5 Hz), 6.83 (1H, td, *J* = 8.5, 2.5 Hz), 6.79 (1H, dd, *J* = 8.5, 4.4 Hz), 5.95–5.85 (1H, m), 5.33 (1H, br d, *J* = 6.8 Hz), 4.17–4.15 (1H, m), 4.12 (2H, t, *J* = 6.7 Hz), 3.82 (2H, t, *J* = 5.7 Hz), 3.24 (1H, t, *J* = 5.7 Hz), 3.12 (4H, q, *J* = 7.3 Hz), 2.55 (3H, s), 2.51–2.49 (1H, m), 2.49 (3H, s), 2.17–2.11 (1H, m), 1.94–1.91 (1H, m), 1.41 (9H, s), 1.36 (3H, t, *J* = 7.3 Hz), 1.35 (3H, t, *J* = 7.3 Hz), 1.13 (3H, d, *J* = 6.8 Hz), 1.12 (3H, d, *J* = 6.8 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.1 (s), 176.5 (s), 166.5 (s), 159.9 (s), 158.3 (s), 155.6 (s), 138.9 (s), 133.3 (s), 131.0 (s), 128.8 (s), 124.2 (d), 120.1 (s), 114.3 (s), 112.8 (s), 112.7 (d), 109.9 (d), 105.1 (d), 79.2 (s), 61.4 (t), 52.7 (t), 52.4 (d), 48.0 × 2 (d), 35.8 (t), 35.6 (t), 33.9 (d), 28.4 × 3 (q), 19.9 ×

2 (q), 14.2 (q), 11.3 (q), 8.6 × 2 (q); HR-ESIMS *m/z* calcd for $C_{35}H_{49}FN_5O_7$ $[M+H]^+$ 670.3616, found 670.3611.

(*S*)-Benzyl 2-(((allyloxy)carbonyl)amino)-4-(isobutyryloxy)butanoate (8): A solution of **3** (284 mg, 0.75 mmol) in $CHCl_3$/TFA (2.5:1, 3.5 mL) was stirred at room temperature for 30 min. The solvent was removed *in vacuo*, and the residue was dissolved in 1,4-dioxane (2.1 mL) together with allyl chloroformate (159 μL, 1.50 mmol). The mixture was stirred at room temperature for 5 min, and saturated $NaHCO_3$ solution (0.70 mL) was added. Then, the mixture was stirred at room temperature for 10 h. Aqueous HCl (1 M, 20 mL) was added, and the whole was extracted with $CHCl_3$ (3 × 10 mL). The combined $CHCl_3$ extracts were washed with brine (10 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. The residue was subjected to column chromatography (silica gel, 5:1:1 $CHCl_3$/hexane/EtOAc) to afford **8** (216 mg, 80%) as a colorless oil: $[\alpha]^{25}_D$ +0.4 (c 0.24, $CHCl_3$); IR (film) ν 3350, 2974, 1730, 1528, 1157 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 7.38–7.32 (5H, m), 5.89 (1H, octet, *J* = 5.5 Hz), 5.89 (1H, d, *J* = 7.6 Hz), 5.30 (1H, d, *J* = 17.3 Hz), 5.21 (1H, d, *J* = 8.7 Hz), 5.18 (2H, d, *J* = 4.2 Hz), 4.56 (2H, d, *J* = 5.5 Hz), 4.53 (1H, dd, *J* = 12.8, 7.6 Hz), 4.17 (1H, dd, *J* = 12.8, 7.0 Hz), 4.09 (1H, dd, *J* = 11.7, 7.0 Hz), 2.48 (1H, sept, *J* = 7.0 Hz), 2.22 (1H, sept, *J* = 7.0 Hz), 2.06 (1H, sept, *J* = 7.0 Hz), 1.136 (3H, d, *J* = 7.0 Hz), 1.134 (3H, d, *J* = 7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.8 (s), 171.7 (s), 155.7 (s), 135.1 (s), 132.5 (d), 128.6 × 2 (d), 128.5 (d), 128.2 × 2 (d), 117.9 (t), 67.4 (t), 65.9 (t), 60.2 (t), 51.5 (d), 33.8 (d), 31.2 (d), 18.9 × 2 (q); HR-ESIMS *m/z* calcd for $C_{19}H_{25}NO_6Na$ $[M+Na]^+$ 386.1580, found 386.1576.

***tert*-Butyl undec-10-enoate (9):** 10-Undecenoic acid (500 mg, 2.7 mmol) was dissolved in THF (0.5 mL) together with *tert*-butanol (1.1 mL, 11.6 mmol) and DMAP (33.2 mg, 0.27 mmol), and EDC·HCl (522 mg, 2.7 mmol) was added at 0 °C. The mixture was stirred at this temperature for 1 h, and then at room temperature for 20 h. Saturated aqueous $NaHCO_3$ (10 mL) was added, and the mixture was extracted with $CHCl_3$ (3 × 10 mL). The combined $CHCl_3$ extracts were washed with brine (10 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. The residue was subjected to column chromatography (silica gel, 20:1 hexane/EtOAc) to afford **9** (355 mg, 55%) as a colorless oil: $[\alpha]^{25}_D$ -3.6 (c 0.18, $CHCl_3$); IR (film) ν 3077, 2978, 2927, 2855, 1732, 1640 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 5.80 (1H, ddt, *J* = 17.1, 10.2, 6.7 Hz), 4.98 (1H, dd, *J* = 17.1, 3.7 Hz), 4.91 (1H, d quint, *J* = 10.2, 1.2 Hz), 2.19 (2H, t, *J* = 7.5 Hz), 2.03 (2H, qt, *J* = 6.7, 1.5 Hz), 1.56 (2H, quint, *J* = 7.5 Hz), 1.44 (9H, s), 1.36 (2H, quint, *J* = 7.5 Hz), 1.28 (8H, br s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.3 (s), 139.1 (d), 114.1 (t), 79.9 (s), 35.6 (t), 33.8 (t), 29.3 × 2 (t), 29.2 (t), 29.0 (t), 28.9 (t), 28.1 × 3 (q), 25.1 (t); HR-ESIMS *m/z* calcd for $C_{15}H_{29}O_2$ $[M+H]^+$ 241.2168, found 241.2168.

(*S*,*E*)-*tert*-Butyl 12-(((1-(benzyloxy)-4-(isobutyryloxy)-1-oxobutan-2-yl)carbamoyl)oxy)dodec-10-enoate (10): Compounds **8** (122 mg, 0.33 mmol) and **9** (161 mg, 0.67 mmol) were dissolved in CH_2Cl_2 (434 μL) together with Grubbs 2nd generation catalyst (28.4 mg, 0.033 mmol), and the mixture was stirred at 55 °C under an atmosphere of argon for 45 h. The solvent was removed *in vacuo*, and the residue was dissolved in CH_2Cl_2. Chromatorex® NH silica gel (500 mg) was added to the solution, and the mixture was stirred at room temperature for 1 h. Insoluble material was filtered off, and the filtrate was concentrated to dryness. The residue was subjected to HPLC (85:15 MeOH/H_2O) to provide **10** (54.9 mg, 29%) as a colorless oil: $[\alpha]^{25}_D$ +2.3 (c 0.29, $CHCl_3$); IR (film) ν 3354, 2974, 2929, 2857, 1730, 1154 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 7.38–7.31 (5H, m), 5.74 (1H, quint, *J* = 7.0 Hz), 5.53 (1H, quint, *J* = 7.0 Hz), 5.37 (1H, d, *J* = 7.9 Hz), 5.17 (2H, dd, *J* = 12.5, 4.5 Hz), 4.53–4.51 (1H, m), 4.49 (2H, d, *J* = 7.0 Hz), 4.16 (1H, dq, *J* = 11.7, 6.9 Hz), 4.08 (1H, dq, *J* = 12.1, 6.9 Hz), 2.47 (1H, sept, *J* = 6.8 Hz), 2.23–2.21 (1H, m), 2.18 (2H, t, *J* = 7.6 Hz), 2.068–2.006 (1H, m), 2.02 (2H, q, *J* = 6.9 Hz), 1.56 (2H, quint, *J* = 7.0 Hz), 1.43 (9H, s), 1.36–1.34 (2H, m), 1.27 (8H, br s), 1.129 (3H, d, *J* = 7.0 Hz), 1.128 (3H, d, *J* = 7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.8 (s), 173.3 (s), 171.8 (s), 155.8 (s), 136.4 (d), 135.1 (s), 128.6 × 2 (d), 128.5 (d), 128.2 × 2 (d), 123.9 (d), 79.8 (s), 67.3 (t), 66.1 (t), 60.2 (t), 51.4 (d), 35.6 (t), 33.8 (d), 32.2 (t), 31.2 (t), 29.25 (t), 29.20 (t), 29.08 (t), 29.04 (t), 28.1 × 3 (q), 25.1 (t), 18.88 (q), 18.79 (q); HR-ESIMS *m/z* calcd for $C_{32}H_{49}NO_8$ Na $[M+Na]^+$ 598.3356, found 598.3350.

(*S*)-*tert*-Butyl 12-(((4-(isobutyryloxy)-1-oxo-1-(pentafluorophenoxy)butan-2-yl)carbamoyl)oxy)dodecanoate (11): 10% Pd/C (5.0 mg) was added to a solution of **10** (36.5 mg, 0.063 mmol) in CH_2Cl_2/EtOAc (2:1, 3.0 mL), and the mixture was stirred at room temperature under an atmosphere of hydrogen for 30 min. The catalyst was filtered off, and the filtrate was concentrated to dryness. The residue was dissolved in CH_2Cl_2 (200 μL) together with pentafluorophenol (35.0 mg, 0.19 mmol) and DCC (13.1 mg, 0.063 mmol). The mixture was stirred at room temperature for 4 h. Saturated aqueous $NaHCO_3$ (10 mL) was added, and the mixture was extracted with $CHCl_3$ (3 × 10 mL). The combined $CHCl_3$ extracts were washed with brine (10 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. The residue was subjected to column chromatography (silica gel, 6:1 hexane/EtOAc) to afford **11** (33.2 mg, 81%) as a colorless oil: $[\alpha]^{25}_D$ -19.7 (c 0.33, MeOH); IR (film) ν 3342, 2975, 2931, 2857, 1730, 1523, 1155, 999 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 5.42 (1H, d, *J* = 8.0 Hz), 4.83 (1H, td, *J* = 8.0, 5.2 Hz), 4.30 (1H, quint, *J* = 5.0 Hz), 4.20 (1H, dq, *J* = 12.8, 5.0 Hz), 4.08 (2H, t, *J* = 6.2 Hz), 2.55 (1H, sept, *J* = 7.0 Hz), 2.42–2.37 (1H, m), 2.24–2.22 (1H, m), 2.19 (2H, t, *J* = 7.6 Hz), 1.62–1.60 (2H, m), 1.57–1.53 (2H, m), 1.43 (9H, s), 1.32–1.25 (12H, m), 1.17 (3H, d, *J* = 7.0 Hz), 1.16 (3H, d, *J* = 7.0 Hz); ^{13}C NMR

(150 MHz, $CDCl_3$) δ 176.9 (s), 173.5 (s), 168.5 (s), 156.1 (s), 141.83 (s), 141.78 (s), 140.1 (s), 137.12 (s), 137.06 × 2 (s), 80.1 (s), 65.9 (t), 60.1 (t), 51.2 (d), 35.6 (t), 33.9 (d), 31.0 (t), 29.5 × 2 (t), 29.4 (t), 29.3 (t), 29.2 (t), 29.1 (t), 28.9 (t), 28.1 × 3 (q), 25.8 (t), 25.1 (t), 18.9 × 2 (q); HR-ESIMS *m/z* calcd for $C_{31}H_{44}F_5NO_8Na$ $[M+Na]^+$ 676.2885, found 676.2906.

(*S*,*Z*)-*tert*-Butyl 12-(((1-(3-((4-((2-(diethylamino)ethyl)carbamoyl)-3,5-dimethyl-1*H*-pyrrol-2-yl)methylene)-5-fluoro-2-oxoindolin-1-yl)-4-(isobutyryloxy)-1-oxobutan-2-yl)carbamoyl)oxy)dodecanoate (12): Compound **11** (16.9 mg, 0.026 mmol) and sunitinib (6.9 mg, 0.017 mmol) were dissolved in DMF (200 μL), and the mixture was stirred at room temperature for two days. The solvent was removed *in vacuo*, and the residue was subjected to column chromatography (silica gel, 20:1 $CHCl_3$/MeOH) to afford **12** (13.7 mg, 61%) as an orange amorphous solid: $[\alpha]^{25}_D$ +36.0 (c 0.38, MeOH); IR (film) ν 3411, 2975, 2928, 2855, 1728, 1154 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 12.68 (1H, s), 8.16 (1H, q, *J* = 4.5 Hz), 8.14–8.11 (1H, m), 7.38 (1H, s), 7.20 (1H, dd, *J* = 8.1, 2.0 Hz), 6.90 (1H, td, *J* = 8.7, 2.0 Hz), 5.95–5.91 (1H, m), 5.74 (1H, d, *J* = 8.7 Hz), 4.27 (2H, t, *J* = 6.4 Hz), 4.06 (2H, t, *J* = 6.8 Hz), 3.71–3.69 (2H, m), 3.07–3.04 (2H, m), 2.94 (4H, quint, *J* = 6.6 Hz), 2.60 (3H, s), 2.51 (3H, s), 2.49–2.43 (1H, m), 2.39–2.34 (1H, m), 2.19 (2H, t, *J* = 7.7 Hz), 2.11–2.06 (1H, m), 1.56–1.52 (2H, m), 1.43 (9H, s), 1.29–1.24 (16H, m), 1.22 (6H, m), 1.12 (3H, d, *J* = 7.0 Hz), 1.11 (3H, d, *J* = 7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.8 (s), 173.4 (s), 169.9 (s), 168.2 (s), 165.8 (s), 159.7 (s), 156.4 (s), 140.5 (s), 133.4 (s), 132.0 (s), 128.3 (s), 126.4 (s), 125.3 (d), 120.7 (s), 117.8 (d), 113.0 (d), 111.1 (s), 104.1 (d), 79.9 (s), 65.5 (t), 60.8 (t), 53.2 (d), 51.5 (t), 47.1 (t), 47.0 (t), 35.9 (t), 35.6 (t), 33.9 (d), 31.8 (t), 29.53 (t), 29.46 (t), 29.33 (t), 29.31 (t), 29.11 (t), 29.06 (t), 29.0 (t), 28.1 × 3 (q), 25.9 (t), 25.1 (t), 18.94 (q), 18.91 (q), 14.4 (q), 11.3 (q), 9.5 (q), 9.4 (q); HR-ESIMS *m/z* calcd for $C_{47}H_{71}FN_5O_9$ $[M+H]^+$ 868.5236, found 868.5256.

(*S*,*Z*)-Pentafluorophenyl 12-(((1-(3-((4-((2-(diethylamino)ethyl)carbamoyl)-3,5-dimethyl-1*H*-pyrrol-2-yl)methylene)-5-fluoro-2-oxoindolin-1-yl)-4-(isobutyryloxy)-1-oxobutan-2-yl)carbamoyl)oxy)dodecanoate (13): A solution of **12** (74.0 mg, 0.085 mmol) in $CHCl_3$/TFA (4:1, 2.0 mL) was stirred at room temperature for 3 h, and the solvent was removed *in vacuo.* The residue was dissolved in CH_2Cl_2 (200 μL) together with pentafluorophenol (49.2 mg, 0.27 mmol) and DMAP (0.14 mg, 0.0011 mmol), and EDC·HCl (37.5 mg, 0.20 mmol) was added at 0 °C. The mixture was stirred at this temperature for 1 h, and then at room temperature for 21 h. Saturated aqueous $NaHCO_3$ (10 mL) was added, and the mixture was extracted with $CHCl_3$ (3 × 10 mL). The combined $CHCl_3$ extracts were washed with brine (10 mL), dried over Na_2SO_4, and filtered, and the solvent was removed *in vacuo*. The residue was subjected to column chromatography (silica gel, 20:1 $CHCl_3$/MeOH) to afford **13** (60 mg, 72%) as a yellow amorphous solid: $[\alpha]^{25}_D$ +43.8 (c

0.23, $CHCl_3$); IR (film) ν 3364, 2927, 2855, 2360, 1791, 1698, 1520 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) δ 11.62 (1H, br s), 8.16 (1H, q, *J* = 4.5 Hz), 8.14–8.12 (1H, m), 7.39 (1H, s), 7.17 (1H, dd, *J* = 8.3, 2.6 Hz), 6.91 (1H, td, *J* = 8.7, 2.6 Hz), 5.95–5.91 (1H, m), 5.72 (1H, d, *J* = 8.3 Hz), 4.27 (2H, t, *J* = 5.8 Hz), 4.07 (2H, t, *J* = 6.4 Hz), 3.89 (2H, br t, *J* = 4.5 Hz), 3.31–3.29 (2H, m), 3.20–3.16 (4H, m), 2.67 (3H, s), 2.65 (2H, t, *J* = 7.5 Hz), 2.60 (3H, s), 2.46–2.41 (1H, m), 2.37–2.31 (1H, m), 2.09–2.06 (1H, m), 1.76 (2H, quint, *J* = 7.5 Hz), 1.639–1.580 (2H, m), 1.444 (3H, t, *J* = 7.2 Hz), 1.441 (3H, t, *J* = 7.2 Hz), 1.33–1.32 (2H, m), 1.32–1.24 (12H, m), 1.13 (3H, d, *J* = 7.2 Hz), 1.09 (3H, d, *J* = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.9 (s), 173.0 (s), 169.8 (s), 168.2 (s), 166.0 (s), 159.7 (s), 156.3 (s), 142.0 × 2 (s), 141.0 (s), 140.3 (s), 138.7 × 2 (s), 137.7 (s), 134.1 (s), 132.0 (s), 128.3 (s), 126.5 (s), 125.4 (d), 119.6 (s), 117.9 (d), 113.1 (d), 111.3 (s), 104.1 (d), 65.5 (t), 60.8 (t), 53.6 (t), 53.1 (d), 48.86 (t), 48.83 (t), 35.5 (t), 33.9 (d), 33.4 (t), 31.8 (t), 29.5 (t), 29.4 (t), 29.28 (t), 29.25 (t), 29.1 (t), 29.0 (t), 28.9 (t), 25.8 (t), 24.8 (t), 18.92 (q), 18.90 (q), 14.7 (q), 11.7 (q), 8.7 × 2 (q); HR-ESIMS *m/z* calcd for $C_{49}H_{61}F_6N_5O_9$ $[M+H]^+$ 978.4452, found 978.4474.

Sunitinib–metastin conjugate (14): Compound **13** (3.7 mg, 0.0038 mmol) and metastin (1.1 mg, 0.00018 mmol) were dissolved in DMF (600 μL), and the mixture was stirred at room temperature for five days. The mixture was passed through a PD-10 desalting column (GE Healthcare Life Sciences) with H_2O as eluent to give a fraction containing the conjugate. The solution was lyophilized by using FDU-2200 (EYELA) to provide conjugate **14** (0.96 mg, 64%); ESI-TOF-MS *m/z* 1031.2981 $[M+8H]^{8+}$, *m/z* 1374.0596 $[M+7H]^{7+}$, *m/z* 1778.1360 $[M+6H]^{6+}$.

Enzymatic Hydrolysis of Compounds 6 and 7

An equivalent molar amount of malic acid was added to compound **6** or **7**. The mixture was dissolved in methanol, and the solution was concentrated to dryness. The residue (each 290 μg) was dissolved in PBS (450 μL, pH 7.4) and the solution was left to stand at 36.6 °C with or without PLE (100 μg, 17 units/mg, SIGMA). The reaction was monitored by injecting a 10 μL aliquot of the reaction mixture into an analytical HPLC equipped with DP-L910W software, using the following conditions: mobile phase, 55:45 MeCN/20 mM sodium acetate buffer; flow rate, 1 mL/min; λ = 431 nm. The t_R values for sunitinib, compound **6**, and compound **7** were 8, 20, and 16 min, respectively.

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