

Isolation and Absolute Configuration of SMTP-0, a Simplest Congener of the SMTP Family Nonlysine-analog Plasminogen Modulators

Keiji Hasumi, Keiko Hasegawa, Yoshikazu Kitano

Received: April 16, 2007 / Accepted: July 12, 2007

© Japan Antibiotics Research Association

Abstract SMTP-0, a new simple congener of the SMTP nonlysine-analog plasminogen modulators, was isolated from a culture of *Stachybotrys microspora*. Based on the physico-chemical data, SMTP-0 was shown to be an enantiomer of the antimicrobial compound stachybotrin B. The absolute configuration of SMTP-0 was determined by the modified Mosher method. The stereochemistry was further confirmed using an epimer of SMTP-0. Unlike most SMTPs with an amino acid side chain linked to the nitrogen atom of the lactam moiety, SMTP-0, which lacks the *N*-linked side chain, showed no plasminogen modulator activity.

Keywords nonlysine-analog plasminogen modulator, absolute configuration, *Stachybotrys microspora*, microbial triprenyl phenols, modified Mosher method

The plasminogen/plasmin system plays crucial roles not only in blood clot lysis but also in various physiological and pathological events, where localized proteolysis is required [1, 2]. Human plasminogen is a single-chain glycoprotein consisting of an *N*-terminal peptide, five kringle domains and a serine protease domain [3]. Two serine proteases, tissue-type plasminogen activator and urokinase type plasminogen activator (u-PA), catalyze the activation of plasminogen by cleaving the Arg⁵⁶¹-Val⁵⁶² bond to yield active serine protease plasmin, which consists of two polypeptide chains that are held together

by disulfide bridges [2]. Circulating plasminogen resists its activation, since it adopts tight, spiral conformation due to intramolecular binding of Lys⁵⁰ and/or Lys⁶² to lysine binding site (aminohexyl site) in the fifth kringle domain [4~6]. Fibrin and cellular receptors bind plasminogen and relax plasminogen conformation to be highly activatable, promoting efficient localized proteolysis [7, 8]. Similarly, lysine analogs bind to plasminogen kringles, resulting in conformational relaxation and enhancement of activation to plasmin [4]. Thus, conformational modulation of plasminogen is important for its activation.

We discovered a variety of novel nonlysine-analog modulators of plasminogen activation. These include a family of microbial triprenyl phenol metabolites designated SMTPs, which enhance both plasminogen activation and plasminogen-fibrin binding, resulting in augmentation of fibrinolysis [9~16]. These effects are strikingly different from the action of lysine analog modulators, which enhance plasminogen activation but inhibit plasminogen-fibrin binding and fibrinolysis. Therefore, SMTPs could be candidates for development of fibrinolysis-promoting agents. In the present paper, we describe the isolation of a new congener, SMTP-0 (Fig. 1), which lacks the side chain of SMTPs and permits determination of its absolute configuration by the modified Mosher method.

Previously, we found that the production of SMTPs by *Stachybotrys microspora* could be selectively enhanced by supplementing the culture medium with an appropriate amine, of which nitrogen atom is to be incorporated as the lactam nitrogen atom in the SMTP molecule [14]. We have improved the method by investigating the composition of production medium and the timing of amine feeding. The essential modification was to restrict the amount of amines in the production medium in the early phase of culture so as to permit cell growth but suppress the

K. Hasumi (Corresponding author), **K. Hasegawa**, **Y. Kitano**:
Department of Applied Biological Science, Tokyo Noko
University, 3-5-8 Saiwaicho, Fuchu-shi, Tokyo 183-8509, Japan,
E-mail: hasumi@cc.tuat.ac.jp

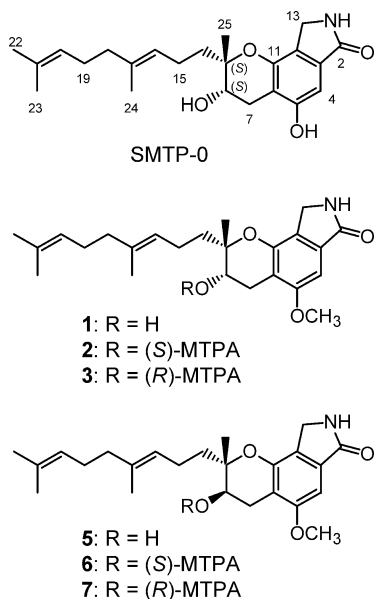


Fig. 1 Absolute structure of **1**, **2**, **3**, **5**, **6** and **7**. SMTP-0, 5-*O*-Me-SMTP-0 (**1**), 8-[*S*- α -methoxy- α -(trifluoromethyl)phenylacetoxyl]-5-*O*-Me-SMTP-0 (**2**), 8-[*R*- α -methoxy- α -(trifluoromethyl)phenylacetoxyl]-5-*O*-Me-SMTP-0 (**3**), 8-*epi*-5-*O*-Me-SMTP-0 (**5**), 8-*epi*-8-[*S*- α -methoxy- α -(trifluoromethyl)phenylacetoxyl]-5-*O*-Me-SMTP-0 (**6**), and 8-*epi*-8-[*R*- α -methoxy- α -(trifluoromethyl)phenylacetoxyl]-5-*O*-Me-SMTP-0 (**7**) are shown.

Table 1 Physico-chemical properties of SMTP-0

Appearance	White amorphous solid
Molecular formula	C ₂₃ H ₃₁ NO ₄
MALDI-TOF-MS (<i>m/z</i>)	
Found (M+H) ⁺ :	386.2599
Calcd:	386.2331 for C ₂₃ H ₃₂ NO ₄
UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ)	215 (55,770), 251 (10,620), 300 (4,000)
IR ν_{max} (neat) cm ⁻¹	3259, 2918, 1666, 1612, 1470, 1360, 1167, 1082
$[\alpha]_{\text{D}}^{25}$	-7.4° (c 0.5, CHCl ₃)

formation of byproducts. SMTP congeners of interest can be produced selectively by adding appropriate amines after accumulation of a triprenyl phenol precursor. SMTP-0 was produced using the improved method as described in the experimental section. From 100 ml of the *S. microspora* IFO 30018 culture, 33.2 mg of purified SMTP-0 was isolated.

The physico-chemical properties of SMTP-0 are shown in Table 1. The NMR and MS results (see Table 2 for NMR data) suggested that the compound retained the backbone structure common to the previously isolated SMTPs

but that it lacked the *N*-linked side chain (Fig. 1). The structural formula of SMTP-0 was identical to that of stachybotrin B, isolated by Xu *et al.* as an antimicrobial agent. However, the optical rotation of SMTP-0 was -7.4° , but that of stachybotrin B was $+39.1^\circ$. The NMR data of stachybotrin B were quite similar to those of SMTP-0 (Table 2). These observations suggest that SMTP-0 is an enantiomer of stachybotrin B, while its absolute configuration has been unknown. So, we tried to determine the absolute configuration of SMTP-0. In the previous studies, we determined the relative stereochemistry of the dihydropyran moiety of SMTP [15]. There remained two possible combinations of absolute configuration at C8 and C9, *i.e.* 8*R*,9*R* and 8*S*,9*S*. The modified Mosher method is known to give an absolute configuration for a chiral carbon atom with a secondary hydroxyl group [18, 19]. Because SMTP-0 has two hydroxyl groups (one attached to the chiral carbon atom at C8 and the other to the aromatic carbon atom at C5), we first converted SMTP-0 to its 5-*O*-Me derivative (**1**) using trimethylsilyldiazomethane [20] (Fig. 1). Next, compound **1** was esterified using *R*-(-)- and *S*-(+)- α -methoxy- α -(trifluoromethyl)phenylacetylchloride to afford 8-[*S*- α -methoxy- α -(trifluoromethyl)phenylacetoxyl]-5-*O*-Me-SMTP-0 (**2**) and 8-[*R*- α -methoxy- α -(trifluoromethyl)phenylacetoxyl]-5-*O*-Me-SMTP-0 (**3**), respectively (Fig. 1). The chemical shift values of ¹H-NMR spectra for compounds **2** and **3** were compared each other (Table 3). Characteristic differences in the chemical shift value ($\Delta\delta = \delta_S - \delta_R$) were observed at the axial proton at C7 ($\Delta\delta = -0.12$), the aromatic proton at C4 ($\Delta\delta = -0.04$), the olefinic proton at C16 ($\Delta\delta = +0.04$) and the methyl protons at C25 ($\Delta\delta = +0.05$), thus indicating an *S* configuration at C8. To exclude ambiguity, we further tried to synthesize Mosher esters using an epimer of SMTP-0. First, alcohol **1** was oxidized with Dess-Martin periodinane [21], affording ketone **4**. Then, ketone **4** was reduced with NaBH₄ to yield racemic alcohol, from which 8-*epi*-5-*O*-Me-SMTP-0 (**5**) was isolated by reverse phase HPLC. Both *S* and *R* Mosher esters (**6** and **7**, respectively) were prepared from **5**. As shown in Table 3, plus and minus signs of the characteristic differences in the chemical shift values between **6** and **7** were opposite as compared with the differences between **2** and **3**. These results support the conclusion that configuration at C8 is *S*. Therefore, the configurations at C8 and C9 were assigned as *S* and *S* (Fig. 1). Because all the SMTP congeners are produced by the same strain of *S. microspora* IFO 30018 and the optical rotation of the congeners with relatively simple *N*-linked side chain, staplablin [-(CH₂)₄COOH] and SMTP-1 [-(CH₂)₂OH], are in the minus values (-11.0° and -7.1° , respectively) [9,

Table 2 NMR spectral data for SMTP-0 and stachybotrin B

No.	SMTP-0		Stachybotrin B	
	δ_C	δ_H	δ_C	δ_H
2	170.24		174.2	
3	131.78		132.5	
4	99.44	6.62 (1H, s)	100.8	6.74 (1H, s)
5	155.93		158.0	
6	111.29		113.5	
7	26.59	2.82 (1H, dd, $J=5.5, 17.4$) 2.45 (1H, dd, $J=7.7, 17.6$)	27.8	2.98 (1H, dd, $J=6, 18$) 2.64 (1H, dd, $J=6, 18$)
8	66.01	3.73 (1H, t, $J=6.4$)	68.4	3.87 (1H, dd, $J=6$)
9	78.52		80.2	
11	148.45		150.2	
12	121.61		124.1	
13	41.95	4.09 (1H, d, $J=16.9$) 4.05 (1H, d, $J=16.9$)	44.2	4.20 (1H, d, $J=18$) 4.23 (1H, d, $J=18$)
14	37.14	1.59 (2H, m)	38.5	1.67 (2H, m)
15	20.93	2.10 (2H, m)	22.6	2.18 (2H, m)
16	124.16	5.11 (1H, t, $J=6.6$)	125.5	5.14 (1H, m)
17	134.18		136.2	
18	39.04	1.91 (2H, m)	40.8	1.95 (2H, m)
19	26.06	1.99 (2H, m)	27.8	2.02 (2H, m)
20	123.98	5.04 (1H, t, $J=7.1$)	125.3	5.05 (1H, m)
21	130.52		132.2	
22	25.27	1.61 (3H, s)	25.8	1.63 (3H, s)
23	17.36	1.53 (3H, s)	17.7	1.55 (3H, s)
24	15.48	1.54 (3H, s)	15.7	1.57 (3H, s)
25	17.95	1.15 (3H, s)	18.2	1.27 (3H, s)

The chemical shift for SMTP-0 is relative to DMSO- d_6 (δ_C 39.5 ppm; δ_H 2.49 ppm) and that for stachybotryin B is from ref. 17. The coupling constant (J) is given in Hz.

11], we believe that the absolute configuration at the dihydropyran moiety of other SMTPs is the same as that of SMTP-0.

SMTP-0 was eventually inactive in enhancing plasminogen activation when it was assayed as described previously [15] at concentrations ranging from 50~500 μ M, whereas SMTP-7, one of most effective congeners, enhanced the activity 10-fold at 65 μ M. This result clearly shows that the *N*-linked side chain of SMTP plays a significant role in exerting plasminogen activation enhancing activity. Although SMTP-0 itself is an inactive compound, it can be a potential precursor for chemical derivatization through introducing a side chain at the lactam nitrogen atom.

SMTP-0 showed antibacterial activity in disk assay against *Corynebacterium ammoniagenes* (IFO 12612) at 100 and 300 μ g/disk (8 mm) with an inhibitory zone of 9

and 9.5 mm, respectively. However, the compound did not affect the growth of *Bacillus subtilis* (IFO 12210) and *Escherichia coli* (IFO 3301) at 300 μ g/disk. SMTP-0 also inhibited the radial growth of the fungus *Rhizomucor pusillus* (IFO 4579) by 40% in a center-point inoculation assay at 100 μ g/disk, while the compound failed to inhibit the growth of *Penicillium notatum* (IAM 1026) at 1 mg/disk.

Experimental

Production and Purification of SMTP-0

S. microspora IFO 30018 was incubated at 25°C for 4 days in a 500-ml Erlenmeyer flask containing 100 ml of a seed medium consisting of glucose 4.0%, soybean meal 0.5%, dry bouillon 0.3%, yeast extract 0.3% and the antifoam

Table 3 ¹H-NMR data for compounds **1**, **2**, **3**, **5**, **6** and **7**

Position	1 (5- <i>O</i> -Me-SMTP-0)		2 (S-MTPA ester of 1)		3 (R-MTPA ester of 1)		$\Delta\delta$ ($\delta_S - \delta_R$)
	δ_H		δ_H		δ_H		
4	6.89	(1H, s)	6.90	(1H, s)	6.94	(1H, s)	-0.04
7	2.96	(1H, dd, $J=5.5, 18.0$)	3.16	(1H, dd, $J=5.3, 18.0$)	3.16	(1H, dd, $J=5.1, 18.0$)	0
	2.76	(1H, dd, $J=5.5, 18.0$)	2.76	(1H, dd, $J=6.6, 18.0$)	2.88	(1H, dd, $J=5.9, 18.0$)	-0.12
8	3.93	(1H, m)	5.29	(1H, dd, $J=5.5, 6.2$)	5.28	(1H, t, $J=5.5$)	0.01
13	4.3	(1H, d, $J=17.2$)	4.27	(2H, s)	4.28	(2H, s)	-0.01
	4.26	(1H, d, $J=17.2$)					0
14	~1.63	(2H, m)	~1.64	(2H, m)	~1.63	(2H, m)	0.01
15	2.13	(2H, m)	2.12	(2H, m)	2.11	(2H, m)	0.01
16	5.08	(1H, m)	5.06	(1H, m)	5.02	(1H, m)	0.04
18	1.94	(2H, m)	1.96	(2H, t, $J=7.7$)	1.96	(2H, t, $J=7.7$)	0
19	2.02	(2H, m)	2.05	(2H, m)	2.04	(2H, m)	0.01
20	5.05	(1H, m)	5.07	(1H, m)	5.07	(1H, m)	0
22	1.65	(3H, s)	1.67	(3H, s)	1.67	(3H, s)	0
23	1.55	(3H, s)	1.57	(3H, s)	1.56	(3H, s)	0.01
24	1.56	(3H, s)	1.59	(3H, s)	1.58	(3H, s)	0.01
25	1.35	(3H, s)	1.28	(3H, s)	1.23	(3H, s)	0.05
5- <i>O</i> -Me	3.85	(3H, s)	3.86	(3H, s)	3.87	(3H, s)	-0.01

MTPA moiety			3.45	(3H, s)	3.49	(3H, s)	
			7.36	(3H, m)	7.30	(2H, t, $J=7.7$)	
			7.48	(2H, d, $J=7.7$)	7.36	(1H, t, $J=7.3$)	
					7.43	(2H, d, $J=7.7$)	
=====							
Position	5 (8- <i>epi</i> -5- <i>O</i> -Me-SMTP-0)		6 (S-MTPA ester of 5)		7 (R-MTPA ester of 5)		$\Delta\delta$ ($\delta_S - \delta_R$)
	δ_H		δ_H		δ_H		
4	6.93	(1H, s)	6.95	(1H, s)	6.9	(1H, s)	0.05
7	2.93	(1H, dd, $J=5.1, 18.0$)	3.06	(1H, dd, $J=5.1, 18.0$)	3.06	(1H, dd, $J=5.1, 18.0$)	0
	2.82	(1H, dd, $J=4.8, 18.0$)	2.97	(1H, dd, $J=5.1, 18.0$)	2.85	(1H, dd, $J=5.1, 18.0$)	0.12
8	3.94	(1H, m)	5.27	(1H, t, $J=5.1$)	5.27	(1H, t, $J=5.5$)	0
13	4.31	(2H, s)	4.28	(1H, d, $J=17.0$)	4.27	(1H, d, $J=17.2$)	0.01
			4.26	(1H, d, $J=17.0$)	4.23	(1H, d, $J=17.2$)	0.03
14	1.78	(2H, m)	1.58	(2H, m)	1.65	(2H, m)	-0.07
15	2.14	(2H, m)	2.03	(2H, m)	2.05	(2H, m)	-0.02
16	5.16	(1H, m)	4.97	(1H, m)	5.03	(1H, m)	-0.06
18	1.98	(2H, m)	1.95	(2H, m)	1.95	(2H, t, $J=7.7$)	0
19	2.06	(2H, m)	2.00	(2H, m)	2.03	(2H, m)	-0.03
20	5.08	(1H, m)	5.07	(1H, m)	5.06	(1H, m)	0.01
22	1.67	(3H, s)	1.67	(3H, s)	1.66	(3H, s)	0.01
23	1.56	(3H, s)	1.53	(3H, s)	1.53	(3H, s)	0
24	1.60	(3H, s)	1.56	(3H, s)	1.56	(3H, s)	0
25	1.29	(3H, s)	1.28	(3H, s)	1.33	(3H, s)	-0.05
5- <i>O</i> -Me	3.87	(3H, s)	3.87	(3H, s)	3.85	(3H, s)	0.02

MTPA moiety			3.44	(3H, s)	3.43	(3H, s)	
			7.26	(2H, t, $J=7.7$)	7.34	(3H, m)	
			7.34	(1H, m)	7.45	(2H, d, $J=7.7$)	
			7.39	(2H, d, $J=7.7$)			

CB442 (Nippon Oil & Fat Co.) 0.01%, pH 5.8. The seed culture (5.0 ml) was transferred to a 500-ml Erlenmeyer flask containing 100 ml of medium consisting of sucrose 5.0%, yeast extract 0.1%, NaNO₃ 0.3%, K₂HPO₄ 0.1%, MgSO₄·7H₂O 0.05%, KCl 0.05%, CoCl₂·6H₂O 0.00025%, FeSO₄·7H₂O 0.0015%, CaCl₂·2H₂O 0.00065% and CB442 0.01%, pH 5.8. The flask was incubated at 25°C on a rotary shaker at 180 rpm. After 96 hours, 100 mg of ammonium chloride was added, and the flask was incubated further for 24 hours. The culture was stopped by adding 200 ml of MeOH. The MeOH extracts were filtered and concentrated to remove MeOH. The residue was extracted thrice with an equal volume of ethyl acetate. The ethyl acetate extracts were combined and concentrated to dryness, dissolved in 3.0 ml of MeOH, treated with Lichrolut® RP-18 (Merck), and subjected to purification by preparative HPLC using an Inertsil PREP-ODS (30×250 mm; GL Science, Tokyo, Japan). The column was developed at a rate of 25 ml/minute at 40°C with 50 mM ammonium acetate in 75% aqueous MeOH. SMTP-0 was eluted at a retention time of 11.5 minutes. Fractions containing SMTP-0 were evaporated to remove MeOH and extracted with ethyl acetate, giving 33.2 mg of purified material.

5-O-Me-SMTP-0 (1)

SMTP-0 (34 mg) in 2.0 ml of methanol-acetonitrile (1 : 9, v/v) was mixed with 200 μl of trimethylsilyldiazomethane (2 M in *n*-hexane) and 40 μl of *N,N*-diisopropylethylamine at room temperature for 24 hours. The mixture was concentrated and passed through a short silica gel column developed with ethyl acetate, affording 27 mg of **1**. MALDI-TOF-MS, *m/z* 399.2410 (M+H)⁺; optical rotation, [α]_D²⁵ -5.9° (*c* 1.0, CHCl₃); ¹H-NMR, see Table 3.

8-[*S*-α-Methoxy-α-(trifluoromethyl)phenylacetoxy]-5-O-Me-SMTP-0 (2)

Compound **1** (12 mg, 30 μmol) in 1.0 ml of dichloromethane was mixed with 10 μl of triethylamine, 2.0 mg of 4-dimethylaminopyridine and 15 mg (60 μmol) of *R*-(-)-α-methoxy-α-(trifluoromethyl)phenylacetylchloride for 24 hours at room temperature. After the mixture was concentrated, the residue was subjected to preparative silica gel TLC (ethyl acetate) to isolate 15 mg of **2**. MALDI-TOF-MS, *m/z* 616.2976 (M+H)⁺; optical rotation, [α]_D²⁵ +18.8° (*c* 0.24, CHCl₃); ¹H-NMR, see Table 3.

8-[*R*-α-Methoxy-α-(trifluoromethyl)phenylacetoxy]-5-O-Me-SMTP-0 (3)

Starting from 12 mg (30 μmol) of **1**, 15 mg of compound **3** was prepared as described above using *S*-(-)-α-methoxy-α-(trifluoromethyl)phenylacetylchloride (15 mg, 60 μmol)

instead of the *R* enantiomer. MALDI-TOF-MS, *m/z* 616.2943 (M+H)⁺; optical rotation, [α]_D²⁵ +41.9° (*c* 0.35, CHCl₃); ¹H-NMR, see Table 3.

8-*epi*-5-O-Me-SMTP-0 (5)

To a solution of alcohol **1** (60 mg, 150 μmol) in 5.0 ml of CH₂Cl₂, 96 mg (226 μmol) Dess-Martin periodinane was added. The reaction mixture was stirred at ambient temperature for 2 hours, and the reaction was quenched with a 1 : 1 solution of saturated aq Na₂S₂O₃ and saturated aq NaHCO₃ (40 ml). The mixture was extracted with CH₂Cl₂ (30 ml, thrice). The combined organic extracts were washed with water, dried over MgSO₄, filtered, and concentrated to yield ketone **4** that was used immediately in the next step. NaBH₄ (15 mg, 400 μmol) was added to a solution of ketone **4** in MeOH (5.0 ml). The reaction mixture was stirred at ambient temperature for 1 hour and was then added brine (50 ml). The mixture was extracted with EtOAc (30 ml, thrice). The combined organic extracts were washed with water, dried over MgSO₄, filtered, and concentrated to yield a 1 : 3 mixture of **1** and **5**. The mixture was subjected to purification by reversed-phase HPLC [Inertsil PREP-ODS, 30×250 mm, GL Science, developed at 12.5 ml/minute with a linear gradient of aq MeOH (80~90% for 40 minutes) containing 0.1% formic acid] to give pure **5** (4.8 mg). MALDI-TOF-MS, *m/z* 400.1589 (M+H)⁺; optical rotation, [α]_D²⁵ -29.5° (*c* 0.39, CHCl₃); ¹H-NMR, see Table 3.

8-*epi*-8-[*S*-α-Methoxy-α-(trifluoromethyl)phenylacetoxy]-5-O-Me-SMTP-0 (6)

A solution of alcohol **5** (2.3 mg, 5.8 μmol) in CH₂Cl₂ (1.0 ml) was mixed with triethylamine (10 μl), 4-dimethylaminopyridine (1.0 mg), and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (5.0 mg, 20 μmol). The reaction mixture was stirred at ambient temperature for 18 hours and was then concentrated. The mixture was subjected to preparative TLC (100% ethyl acetate) to afford purified **6** (3.2 mg). MALDI-TOF-MS, *m/z* 616.2969 (M+H)⁺; optical rotation, [α]_D²⁵ -47.8° (*c* 0.25, CHCl₃); ¹H-NMR, see Table 3.

8-*epi*-8-[*R*-α-Methoxy-α-(trifluoromethyl)phenylacetoxy]-5-O-Me-SMTP-0 (7)

Starting from 2.3 mg (5.8 μmol) of alcohol **5**, 3.0 mg of compound **7** was prepared as described above using *S*-(-)-α-methoxy-α-(trifluoromethyl)phenylacetylchloride (5.0 mg, 20 μmol) instead of the *R* enantiomer. MALDI-TOF-MS, *m/z* 616.2972 (M+H)⁺; optical rotation, [α]_D²⁵ -38.4° (*c* 0.25, CHCl₃); ¹H-NMR, see Table 3.

General

UV spectrum was measured in MeOH on a model 320 spectrometer (Hitachi, Tokyo, Japan) and IR spectrum on a JIR-WINSPEC spectrometer (JEOL, Tokyo, Japan) with NaCl. MALDI-TOF-MS spectrum was taken on a Voyager DE STR spectrometer (Applied Biosystem, CA, USA) using α -cyano-4-hydroxycinnamic acid as a matrix. NMR spectra were measured in DMSO- d_6 or $CDCl_3$ on an EX-270 or an Alpha-600 spectrometer (JEOL). Optical rotation was measured on a model DIP-360 (JASCO, Tokyo Japan) in $CHCl_3$.

Acknowledgments The authors thank Teppei Miura for chemical synthesis of the SMTP-0 derivatives.

References

- Carmeliet P, Collen D. Molecular genetics of the fibrinolytic and coagulation systems in haemostasis, thrombogenesis, restenosis and atherosclerosis. *Curr Opin Lipidol* 8: 118–125 (1997)
- Kolev K, Machovich R. Molecular and cellular modulation of fibrinolysis. *Thromb Haemost* 89: 610–621 (2003)
- Forsgren M, Raden B, Israelsson M, Larsson K, Heden LO. Molecular cloning and characterization of a full-length cDNA clone for human plasminogen. *FEBS Lett* 213: 254–260 (1987)
- Marshall JM, Brown AJ, Ponting CP. Conformational studies of human plasminogen and plasminogen fragments: evidence for a novel third conformation of plasminogen. *Biochemistry* 33: 3599–3606 (1994)
- An SS, Carreno C, Marti DN, Schaller J, Alberico F, Llinas M. Lysine-50 is a likely site for anchoring the plasminogen N-terminal peptide to lysine-binding kringle. *Protein Sci* 7: 1960–1969 (1998)
- Cockell CS, Marshall JM, Dawson KM, Cederholm-Williams SA, Ponting CP. Evidence that the conformation of unliganded human plasminogen is maintained *via* an intramolecular interaction between the lysine-binding site of kringle 5 and the N-terminal peptide. *Biochem J* 333: 99–105 (1998)
- Nesheim, ME, Fredenburgh JC, Larsen GR. The dissociation constants and stoichiometries of the interactions of Lys-plasminogen and chloromethyl ketone derivatives of tissue plasminogen activator and the variant Δ FEIX with intact fibrin. *J Biol Chem* 265: 21541–21548 (1990)
- Hajjar KA, Nacman RL. Endothelial cell-mediated conversion of Glu-plasminogen to Lys-plasminogen. Further evidence for assembly of the fibrinolytic system on the endothelial cell surface. *J Clin Invest* 82: 1769–1778 (1988)
- Shinohara C, Hasumi K, Hatsumi W, Endo A. Staplabin, a novel fungal triprenyl phenol which stimulates the binding of plasminogen to fibrin and U937 cells. *J Antibiot* 49: 961–966 (1996)
- Takayasu R, Hasumi K, Shinohara C, Endo A. Enhancement of fibrin binding and activation of plasminogen by staplabin through induction of a conformational change in plasminogen. *FEBS Lett* 418: 58–62 (1997)
- Kohyama T, Hasumi K, Hamanaka A, Endo A. SMTP-1 and -2, novel analogs of staplabin produced by *Stachybotrys microspora* IFO30018. *J Antibiot* 50: 172–174 (1997)
- Hasumi K, Ohya S, Kohyama T, Ohsaki Y, Takayasu R, Endo A. Isolation of SMTP-3, -4, -5 and -6, novel analogs of staplabin, and their effects on plasminogen activation and fibrinolysis. *J Antibiot* 51: 1059–1068 (1998)
- Hu W, Ohya S, Hasumi K. Activation of fibrinolysis by SMTP-7 and -8, novel staplabin analogs with a pseudosymmetric structure. *J Antibiot* 53: 241–247 (2000)
- Hu W, Ohya S, Narasaki R, Hasumi K. Selective production of staplabin and SMTPs in cultures of *Stachybotrys microspora* fed with precursor amines. *J Antibiot* 54: 962–966 (2001)
- Hu W, Kitano Y, Hasumi K. SMTP-4D, -5D, -6D, -7D and -8D, a new series of the non-lysine-analog plasminogen modulators with a D-amino acid moiety. *J Antibiot* 56: 832–837 (2003)
- Ohya S, Harada T, Chikanishi T, Miura Y, Hasumi K. Nonlysine-analog plasminogen modulators promote autocatalytic generation of plasmin(ogen) fragments with angiotatin-like activity. *Eur J Biochem* 271: 809–820 (2004)
- Xu X, De Guzman FS, Gloer JB, Shearer CA. Stachybotrins A and B: novel bioactive metabolites from a brackish water isolate of the fungus *Stachybotrys* sp. *J Org Chem* 57: 6700–6703 (1992)
- Ohtani I, Kusumi T, Kashman Y, Kakisawa H. High-field FT NMR application of Mosher's method. The absolute configurations of marine terpenoids. *J Am Chem Soc* 113: 4092–4096 (1991)
- Dale JA, Dull DL, Mosher HS. α -Methoxy- α -trifluoromethylphenylacetic acid, a versatile reagent for the determination of enantiomeric composition of alcohols and amines. *J Org Chem* 34: 2543–2549 (1969)
- Aoyama T, Terasawa S, Sudo K, Shioiri T. New methods and reagents in organic synthesis. 46. Trimethylsilyldiazomethane: a convenient reagent for the O-methylation of phenols and enols. *Chem Pharm Bull* 32: 3759–3760 (1984)
- Dess DB, Martin JC. A useful 12-I-5 triacetoxypiperidine (the Dess-Martin periodinane) for the selective oxidation of primary or secondary alcohols and a variety of related 12-I-5 species. *J Am Chem Soc* 113: 7277–7287 (1991)