Synthesis and Evaluation of Water-Soluble Non-Prodrug Analogs of Docetaxel Bearing *sec*-Aminoethyl Group at the C-10 Position

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To develop non-prodrugs of taxoids with satisfactory stability *in vivo*, high water-solubility, and potent antitumor activity, we prepared several 10-*O*-*sec*-aminoethyl docetaxel analogs (3) and evaluated their cytotoxicity against mouse leukemia and human tumor cell lines, microtubule disassembly-inhibitory activity, and water-solubility. These analogs were synthesized from the 10-*O*-allyl baccatin derivatives (5a—c) using the β-lactam synthon method. Among these analogs, the 10-*O*-(2-morpholinoethyl) (18, 21) and 10-*O*-(2-thiomorpholinoethyl) (19, 24) analogs exhibited cytotoxicity comparable or superior to that of docetaxel (2). In addition, the methanesulfonic acid salt (18a) had a high water-solubility.

Key words docetaxel; paclitaxel; non-prodrug; cytotoxicity; microtubule disassembly-inhibitory activity; sec-aminoethyl group

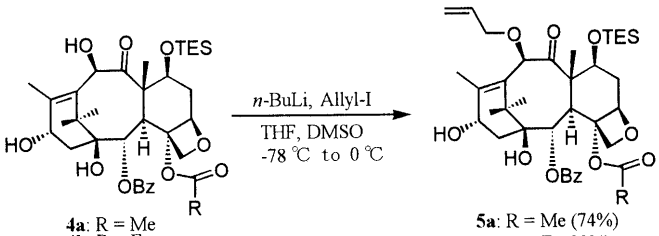
Paclitaxel (**1**, Taxol®),[1] a diterpene natural product isolated by Wani *et al.* from *Taxus brevifolia*, has exceptional efficacy in cancer chemotherapy and was approved by the Food and Drug Administration (FDA) for the treatment of advanced ovarian and breast cancer in December 1992 and April 1994, respectively. Docetaxel (**2**, Taxotere®),[2] a synthetic paclitaxel analog, was approved by the FDA for the treatment of breast cancer in May 1996.[3] Docetaxel has slightly better activity than paclitaxel in several *in vivo* tumor models.[4] Both taxoids possess a unique mechanism of action as promoters of tubulin assembly and inhibitors of microtubule disassembly.[5]

Paclitaxel and docetaxel have impressive efficacy against solid tumors. However, because of their poor water-solubility and poor injectability detergent (Cremophor EL or Tween 80) is necessary for dissolving both taxoids for injection, and these detergents may induce untoward hypersensitivity reactions (hypotension, bronchospasm, urticaria, *etc.*) in patients.[6] To solve the problem of low water-solubility, several research groups have synthesized and evaluated water-soluble taxoids such as esterase- or phosphatase-cleavable prodrugs.[7] Both the C-2′ and C-7 hydroxy functionalities were initially utilized for prodrug synthesis. However, these prodrugs are liable to exhibit unstable efficacy because of variation in the enzymatic activity among patients. Therefore, it is important to develop non-prodrugs of taxoids with satisfactory stability *in vivo*, high water-solubility, and potent antitumor activity. Kant *et al.* have recently reported that the C-10 site has steric latitude for expression of the cytotoxicity.[8] On that basis, we designed water-soluble non-prodrug analogs of docetaxel bearing a *sec*-aminoethyl group at the C-10 position.

In this paper, we report the synthesis and biological activity of the 10-*O*-*sec*-aminoethyl docetaxel analogs (**3**, Fig. 1), which contain further C-4 position and C-13 side chain modifications, and the water-solubility of the methanesulfonic acid salts (**18a**, **19a**). The decision to prepare C-4 and C-13 modified analogs was based on the report that the replacement of the 3′-phenyl, 2′-hydroxy, and/or 4-*O*-Ac moiety with 3′-furyl, 2′,2′-difluoro, and 4-*O*-propionyl/butyryl could further enhance the *in vitro* biological activity.[9]

Chemistry

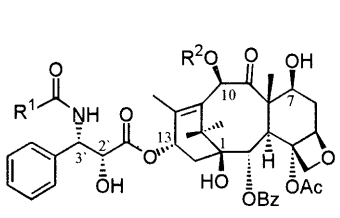
Nine analogs (Table 1) were synthesized from the 10-



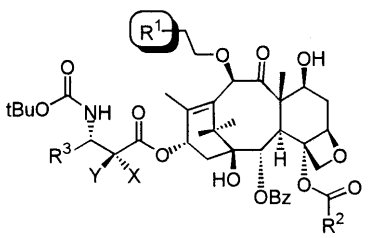
4a: R = Me
4b: R = Et
4c: R = Pr

5a: R = Me (74%)
5b: R = Et (82%)
5c: R = Pr (86%)

Chart 1



Paclitaxel (**1**: R^1 = Ph, R^2 = Ac)
Docetaxel (**2**: R^1 = *t*-BuO, R^2 = H)



3: R^1 = *sec*-amino group; R^2 = Me, Et, or Pr
R^3 = Ph, or 2-furyl; X = OH, Y = H, or X = Y = F

Fig. 1

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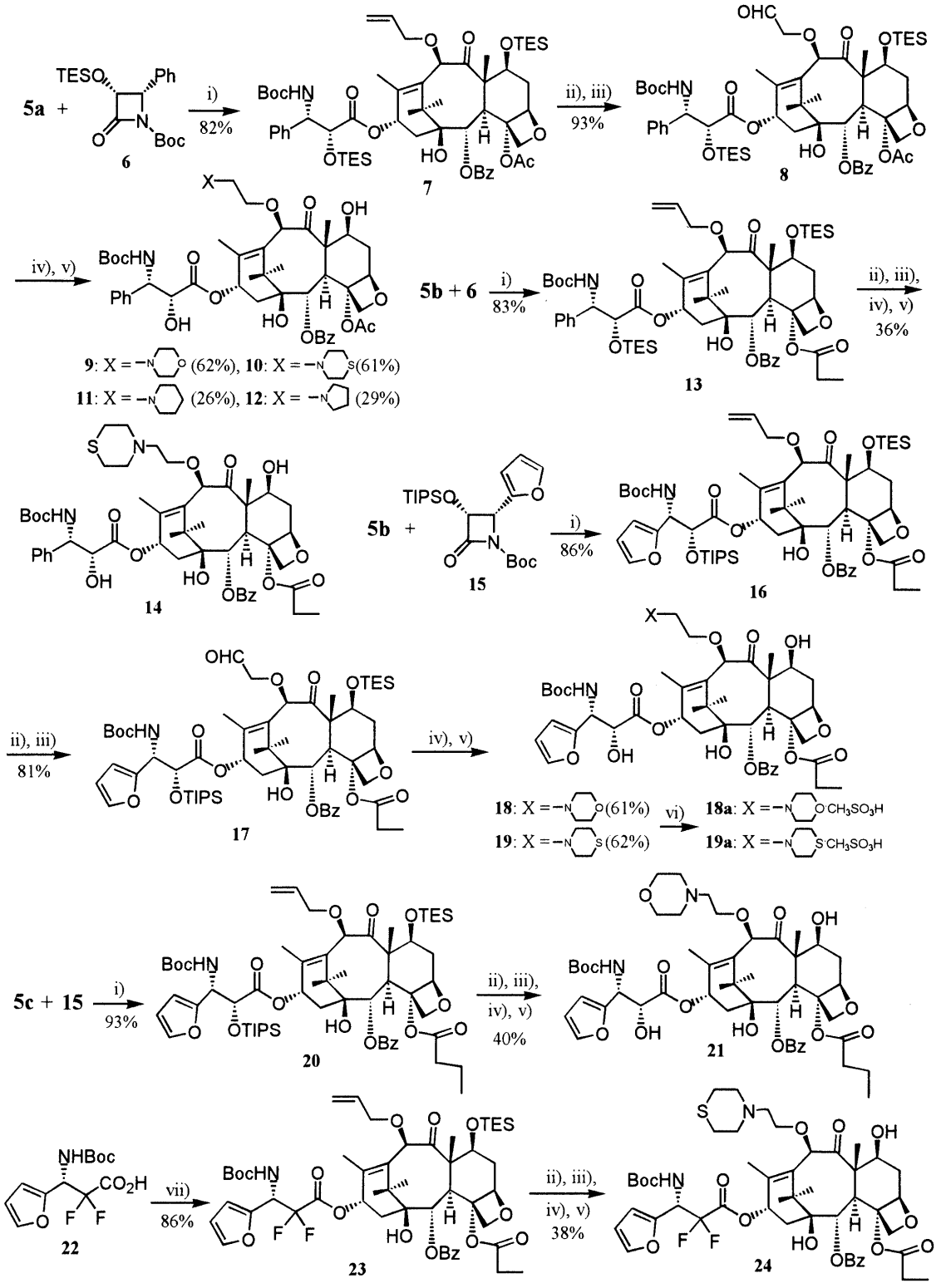
O-allyl baccatin derivatives (**5a**—**c**) using the *β*-lactam synthon method.[10]

The synthesis of key intermediates (**5a**—**c**) is shown in Chart 1. The reaction of 7-triethylsilyl-10-deacetylbaccatin III (**4a**)[11] with 1.5 eq of *n*-BuLi followed by the addition of a dimethyl sulfoxide (DMSO) solution of allyl iodide (1.5 eq) gave compound **5a** in good yield. Similarly, compounds **5b** and **5c** were synthesized from the 4-*O*-acyl baccatin derivatives (**4b**) and (**4c**),[12] respectively

The synthetic route to the 10-*O*-*sec*-aminoethyl docetaxel analogs is shown in Chart 2. Coupling of compound **5a** with the *β*-lactam (**6**) was performed ac-



Reagents and Conditions: i) LiHMDS, THF, -45 °C; ii) OsO_4 (cat.), NMO, acetone, H_2O, rt; iii) $NaIO_4$, MeOH, H_2O, rt; iv) *sec*-amine, AcOH, $NaBH_3CN$, EtOH, rt; v) 48% HF, pyridine, CH_3CN, rt, or HF-Py, pyridine, rt; vi) CH_3SO_3H, 1,4-dioxane, H_2O; vii) **5b**, DPC, DMAP, toluene, 80 °C.

Chart 2

Table 1. Physical Properties of 10-*O*-*sec*-Aminoethyl Docetaxel Analogs

Compound	mp (°C) (dec.)	$[\alpha]_D^{25}$ [a]	MS *m/z* (MH^+)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
9	143—147	−51.7° (*c*=0.54)	921	$C_{49}H_{64}N_2O_{15}\cdot 3/2H_2O$	62.08 (62.31)	7.12 6.88	2.95 2.82)
10	152—156	−52.2° (*c*=0.55)	937	$C_{49}H_{64}N_2O_{14}S\cdot H_2O$	61.62 (61.83)	6.96 6.68	2.93 2.78)
11	134—140	−55.2° (*c*=0.23)	920	$C_{50}H_{66}N_2O_{14}\cdot 2H_2O$	62.87 (63.00)	7.39 7.15	2.93 2.74)
12	136—142	−65.0° (*c*=0.20)	905	$C_{49}H_{64}N_2O_{14}\cdot 3/2H_2O$	63.14 (62.99)	7.24 7.08	3.00 2.83)
14	142—146	−47.0° (*c*=0.30)	951	$C_{50}H_{66}N_2O_{14}S\cdot 3/2H_2O$	61.40 (61.55)	7.10 7.08	2.86 3.05)
18	139—142	−59.5° (*c*=0.20)	925	$C_{48}H_{64}N_2O_{16}\cdot 3/2H_2O$	60.55 (60.74)	7.09 6.85	2.94 2.84)
19	145—148	−62.4° (*c*=0.25)	941	$C_{48}H_{64}N_2O_{15}S\cdot 3/2H_2O$	59.55 (59.45)	6.97 6.78	2.89 2.72)
21	135—137	−56.4° (*c*=0.42)	939	$C_{49}H_{66}N_2O_{16}\cdot 3/2H_2O$	60.92 (61.19)	7.20 7.06	2.90 2.65)
24	136—139	−41.7° (*c*=0.60)	961	$C_{48}H_{62}F_2N_2O_{14}S\cdot 5/2H_2O$	57.31 (57.32)	6.71 6.85	2.78 2.62)

a) Chloroform was used as the solvent.

Table 2. Biological Activity of 10-*O*-*sec*-Aminoethyl Docetaxel Analogs

Entry	Compound	Cytotoxicity GI_{50} (ng/ml)[a]					Tubulin[b] IC_{50}/IC_{50} (**1**)
		P388	PC-6	PC-12	SBC-3	SBC-3/ADM	
1	Paclitaxel (**1**)	25.3	4.64	172	2.14	789	1
2	Docetaxel (**2**)	5.30	1.72	49.7	0.494	280	0.44
3	**9**	24.1	4.79	215	ND	ND	0.65
4	**10**	2.90	2.54	65.4	1.39	319	ND
5	**11**	74.6	6.93	>1000	38.8	>1000	0.42
6	**12**	171	5.38	>1000	115	>1000	0.44
7	**14**	4.21	2.25	4.06	0.555	44.7	ND
8	**18**	2.21	2.08	5.89	1.52	12.1	0.55
9	**19**	1.00	0.84	5.58	0.326	8.03	ND
10	**21**	1.82	2.62	9.37	1.74	12.0	0.60
11	**24**	3.85	3.64	6.68	2.00	14.9	ND
12	**18a**	3.00	2.86	6.27	0.854	11.3	ND
13	**19a**	1.25	1.45	6.01	0.425	9.05	ND

a) The concentration of a compound inhibiting by 50% (GI_{50}) of the growth of cell lines [mouse leukemia (P388), human lung cancer cell lines (PC-6, PC-12, SBC-3), and an adriamycin (ADM)-resistant cancer cell line (SBC-3/ADM)]. b) IC_{50} represents the concentration of an agent leading to 50% inhibition of the rate of microtubule disassembly. IC_{50} (**1**) is the IC_{50} value of paclitaxel [2.6 μM (mean)] in the same assay.

cording to the method of Holton[10e] and gave the desired product (**7**). Oxidation of **7** with OsO_4 followed by cleavage with $NaIO_4$ gave the aldehyde (**8**) in high yield. Finally, compound **8** was subjected to reductive amination[13] with various *sec*-amines followed by standard desilylation to give the 10-*O*-*sec*-aminoethyl docetaxel analogs (**9**—**12**). The 4-*O*-propionyl analog (**14**) was synthesized from **5b** and **6** in a similar manner. The reaction of compounds **5b, c** with the β-lactam (**15**)[14] in the presence of lithium bis(trimethylsilyl)amide (LiHMDS) gave the corresponding coupling products (**16** and **20**, respectively). Following the procedure described for **9**—**12**, compounds **16** and **20** were converted to 3′-(2-furyl)-4-*O*-propionyl analogs (**18**, **19**) and a 3′-(2-furyl)-4-*O*-butyryl analog (**21**) in moderate yields. The preparation of the methanesulfonic acid salts (**18a**, **19a**) was carried out by the method of Stella *et al.*[7b] The 2′,2′-difluoro analog (**24**) was obtained from the coupling product (**23**), which was synthesized by esterification of **5b** with the α,α-difluorinated carboxylic acid (**22**).[9d] Physical properties of the 10-*O*-*sec*-aminoethyl docetaxel analogs are presented in Table 1.

Results (Biological Activity and Water Solubility) and Discussion

Biological activities of the 10-*O*-*sec*-aminoethyl docetaxel analogs were evaluated in two assay systems, *i.e.*, *in vitro* cytotoxicity against five cell lines (P388, PC-6, PC-12, SBC-3, SBC-3/ADM) and microtubule disassembly-inhibitory activity. In order to obtain more meaningful comparisons of relative activities, paclitaxel (**1**) and docetaxel (**2**) were tested as positive controls. The results are presented in Table 2.

A *sec*-amino group at the C-10 position of analogs (**9**—**12**) remarkably influenced the cytotoxicity (entries 3—6). The piperidino (**11**) and pyrrolidinyl (**12**) analogs were about 14—32 times less active than docetaxel (**2**)

Table 3. Estimated Water-Solubility of **18a** and **19a**

Entry	Compound	Estimated water solubility (μg/ml)[a]
1	Paclitaxel (**1**)	35[b]
2	Docetaxel (**2**)	47[b]
3	**18a**	414
4	**18a**	13000 (pH 4.3)
5	**19a**	391

a) The water solubility of each compound was determined using the UV assay. *b*) From Ringel I. R., Horwitz S. B., *J. Natl. Cancer Inst.*, **83**, 288 (1991).

against P388 and absolutely inactive against PC-12 (resistant cancer cell line expressing P-glycoprotein) and SBC-3/ADM. Surprisingly the microtubule disassembly-inhibitory activity of compounds **11** and **12** was of the same order as that of docetaxel. The morpholino analog (**9**) was about 3—5 times less active than docetaxel against P388, PC-6, and PC-12, while the thiomorpholino analog (**10**) retained the activity of docetaxel against all test cell lines. The 4-*O*-propionyl analog (**14**), which was modified at the C-4 position of **10**, exhibited greater activity than docetaxel against PC-12 and SBC-3/ADM (entry 7).

The 4-modified analogs (**18**, **19**, **21**, and **24**) bearing a non-docetaxel side chain showed in a significant increase of activity against resistant cancer cell lines (entries 8—10). For example, the 3′-(2-furyl)-4-*O*-propionyl analog (**19**) proved to be about 12—40 times more active than the 3′-phenyl-4-*O*-acetyl analog (**10**) against PC-12 and SBC-3/ADM. Analog **24**, which contains the 2′,2′-difluoro-3′-(2-furyl) side chain developed in our laboratory, had similar activity against PC-12 to the analog (**19**) bearing a 3′-(2-furyl)-2′-hydroxy side chain (entry 11). Furthermore, the methanesulfonic acid salts (**18a**, **19a**) showed fairly good activity, and **19a** exhibited more potent activity than docetaxel (entries 12, 13).

The water-solubility data for **18a** and **19a** are shown in Table 3. Both salts exhibited increased water-solubility when compared to paclitaxel (**1**) or docetaxel (**2**), and compound **18a** had a high water-solubility (13000 μg/ml) at pH 4.3.

In conclusion, it has become apparent that several 10-*O*-*sec*-aminoethyl docetaxel analogs exhibit comparable or superior cytotoxic activity to docetaxel (**2**) and that the methanesulfonic acid salt (**18a**) possesses sufficient water-solubility. Further investigations into the *in vivo* antitumor activities of these analogs are actively under way in our laboratory.

Experimental

All melting points were found using a Yanaco MP-S3 or MP-500D apparatus and are uncorrected. IR spectra were obtained on a Hitachi 270-300 IR spectrophotometer. Mass spectra were recorded on a JEOL JMS-HX-100, AX505W, or JMS-D300 spectrometer. ^{1}H-NMR spectra were taken at 400 MHz with a JEOL JNM-EX400 spectrometer; all values are reported in ppm (δ) downfield from $(CH_3)_4Si$. Elemental analyses were obtained on a Heraeus CHN-O-Rapid or a Perkin-Elmer 2400CHN instrument. Optical rotations were measured with a Horiba SEPA-200 polarimeter. Merck Silica gel (230—400 mesh) was used for column chromatography. Preparative thin-layer chromatography (preparative TLC) was performed by using silica gel (150A 1.0 mm thickness; PLK5F Whatmann).

10-*O*-Allyl-10-deacetyl-7-*O*-triethylsilylbaccatin III (5a) (General Allylation Procedure) A 1.64 M *n*-hexane solution of *n*-BuLi (0.277 ml) was added dropwise to a solution of **4a** (200 mg, 0.303 mmol) in tetrahydrofuran (THF, 2 ml) at −78 °C under a nitrogen atmosphere. After 15 min, a solution of allyl iodide (0.041 ml, 0.454 mmol) in DMSO (0.5 ml) was added, and stirring was continued for 3 h at 0 °C. The reaction mixture was poured into cold aqueous NH_4Cl solution, and extracted with AcOEt. The extract was washed with brine, then dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (2% MeOH/$CHCl_3$) to give compound **5a** (156 mg, 74%) as a colorless crystalline solid. mp 205—209 °C. MS (FAB) *m/z*: 699 (MH^+). $[\alpha]_D^{26}$ −53.1° (*c*=1.0, $CHCl_3$). *Anal.* Calcd for $C_{38}H_{54}O_{10}Si$: C, 65.30; H, 7.79. Found: C, 65.28; H, 7.79. IR (KBr): 3488, 2956, 2884, 1724 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.50—0.64 (m, 6H), 0.95 (t, 9H, *J*=7 Hz), 1.06 (s, 3H), 1.20 (s, 3H), 1.67 (s, 3H), 1.85—1.92 (m, 1H), 2.08 (s, 3H), 2.26—2.29 (m, 2H), 2.28 (s, 3H), 2.46—2.53 (m, 1H), 3.88 (d, 1H, *J*=7 Hz), 4.01—4.10 (m, 2H), 4.15 (d, 1H, *J*=8 Hz), 4.30 (d, 1H, *J*=8 Hz), 4.43 (dd, 1H, *J*=10, 7 Hz), 4.87—4.90 (m, 1H), 4.96 (d, 1H, *J*=8 Hz), 5.03 (s, 1H), 5.20 (dd, 1H, *J*=10, 1.5 Hz), 5.32 (dd, 1H, *J*=17, 1.5 Hz), 5.61 (d, 1H, *J*=7 Hz), 5.95—6.04 (m, 1H), 7.47 (t, 2H, *J*=8 Hz), 7.60 (t, 1H, *J*=8 Hz), 8.10 (d, 2H, *J*=8 Hz).

10-*O*-Allyl-4,10-dideacetyl-4-*O*-propionyl-7-*O*-triethylsilylbaccatin III (**5b**): Colorless crystalline solid, mp 204—207 °C. MS (FAB) *m/z*: 713 (MH^+). $[\alpha]_D^{26}$ −45.9° (*c*=1.1, $CHCl_3$). *Anal.* Calcd for $C_{39}H_{56}O_{10}Si$: C, 65.70; H, 7.92. Found: C, 65.54; H, 8.00. IR (KBr): 3496, 1724 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.50—0.65 (m, 6H), 0.95 (t, 9H, *J*=7 Hz), 1.06 (s, 3H), 1.20 (s, 3H), 1.68 (s, 3H), 1.85—1.92 (m, 1H), 2.07 (s, 3H), 2.26 (d, 2H, *J*=8 Hz), 2.47—2.57 (m, 1H), 2.58—2.67 (m, 2H), 3.88 (d, 1H, *J*=7 Hz), 4.05—4.07 (m, 2H), 4.15 (d, 1H, *J*=8 Hz), 4.29 (d, 1H, *J*=8 Hz), 4.44 (dd, 1H, *J*=10, 7 Hz), 4.88—4.92 (m, 2H), 5.04 (s, 1H), 5.20 (dd, 1H, *J*=10, 1.5 Hz), 5.31 (dd, 1H, *J*=17, 1.5 Hz), 5.60 (d, 1H, *J*=7 Hz), 5.95—6.04 (m, 1H), 7.46 (t, 2H, *J*=8 Hz), 7.59 (t, 1H, *J*=8 Hz), 8.10—8.12 (m, 2H).

10-*O*-Allyl-4-*O*-butyryl-4,10-dideacetyl-7-*O*-triethylsilylbaccatin III (**5c**): Colorless crystalline solid, mp 200—204 °C. MS (FAB) *m/z*: 727 (MH^+). $[\alpha]_D^{26}$ −41.2° (*c*=1.2, $CHCl_3$). *Anal.* Calcd for $C_{40}H_{58}O_{10}Si$: C, 66.09; H, 8.04. Found: C, 66.15; H, 8.16. IR (KBr): 3504, 1718 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.50—0.65 (m, 6H), 0.96 (t, 9H, *J*=7 Hz), 1.05 (t, 3H, *J*=7 Hz), 1.06 (s, 3H), 1.20 (s, 3H), 1.67 (s, 3H), 1.78 (q, 2H, *J*=7 Hz), 1.82—1.91 (m, 1H), 2.06 (s, 3H), 2.26 (d, 2H, *J*=8 Hz), 2.47—2.57 (m, 1H), 2.55 (t, 2H, *J*=7 Hz), 3.88 (d, 1H, *J*=7 Hz), 4.01—4.13 (m, 2H), 4.15 (d, 1H, *J*=8 Hz), 4.29 (d, 1H, *J*=8 Hz), 4.43 (dd, 1H, *J*=10, 7 Hz), 4.87—4.92 (m, 2H), 5.03 (s, 1H), 5.19 (dd, 1H, *J*=10, 1.5 Hz), 5.31 (dd, 1H, *J*=17, 1.5 Hz), 5.60 (d, 1H, *J*=7 Hz), 5.95—6.04 (m, 1H), 7.46 (t, 2H, *J*=8 Hz), 7.59 (t, 1H, *J*=8 Hz), 8.10—8.12 (m, 2H).

10-*O*-Allyl-13-*O*-[(2*R*,3*S*)-3-(*tert*-butoxycarbonylamino)-3-phenyl-2-(triethylsilyloxy)propionyl]-10-deacetyl-7-*O*-triethylsilylbaccatin III (7) A 1 M THF solution of LiHMDS (0.572 ml) was added dropwise to a solution of **5a** (100 mg, 0.143 mmol) and **6** (108 mg, 0.286 mmol) in THF (2 ml) at −45 °C under a nitrogen atmosphere. After 15 min, the reaction mixture was poured into cold aqueous NH_4Cl solution, and extracted with AcOEt. The extract was washed with brine, then dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by preparative TLC (20% AcOEt/*n*-hexane) to give **7** (126 mg, 82%) as a colorless crystalline solid, mp 109—114 °C. MS (FAB) *m/z*: 1076 (MH^+). $[\alpha]_D^{24}$ −22.3° (*c*=1.0, $CHCl_3$). *Anal.* Calcd for $C_{58}H_{85}NO_{14}Si_2$: C, 64.71; H, 7.96; N, 1.30. Found: C, 64.42; H, 8.13; N, 1.31. IR (KBr): 3452, 2956, 1757, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.31—0.52 (m, 6H), 0.54—0.62 (m, 6H), 0.79 (t, 9H, *J*=7 Hz), 0.96 (t, 9H, *J*=7 Hz), 1.24 (s, 6H), 1.32 (s, 9H), 1.69 (s, 3H), 1.90 (s, 3H), 2.15—2.22 (m, 1H), 2.34—2.41 (m, 1H), 2.45—2.54 (m, 1H), 2.53 (s, 3H), 3.85 (d, 1H, *J*=7 Hz), 4.00—4.13 (m, 2H), 4.19 (d, 1H, *J*=8 Hz), 4.31 (d, 1H, *J*=8 Hz), 4.41 (dd, 1H, *J*=10, 7 Hz), 4.56 (s, 1H), 4.95 (d, 1H, *J*=8 Hz), 4.98 (s, 1H), 5.20 (dd, 1H, *J*=10, 1.5 Hz), 5.29 (br, 1H), 5.32 (dd, 1H, *J*=17, 1.5 Hz), 5.47 (br, 1H), 5.68 (d, 1H, *J*=7 Hz), 5.94—6.02 (m, 1H), 6.31 (t, 1H, *J*=8 Hz), 7.27—7.31 (m, 3H), 7.37 (t, 2H, *J*=8 Hz), 7.48 (t, 2H, *J*=8 Hz), 7.58 (t, 1H, *J*=8 Hz), 8.12 (d, 2H, *J*=8 Hz).

13-*O*-[(2*R*,3*S*)-3-(*tert*-Butoxycarbonylamino)-3-phenyl-2-(triethylsilyloxy)propionyl]-10-deacetyl-10-*O*-formylmethyl-7-*O*-triethylsilylbaccatin III (8) A catalytic amount of OsO_4 (2.5 mg) was added to a solution of **7** (538 mg, 0.500 mmol) and *N*-methylmorpholine *N*-oxide (NMO) (293 mg, 2.50 mmol) in acetone (10 ml) and H_2O (5 ml) at room temperature. After 1 h, saturated aqueous sodium thiosulfate was added, and stirring was continued for 15 min at 0 °C. The mixture was extracted

with three portions of AcOEt. The combined organic layer was washed with saturated aqueous NH_4Cl and brine, then dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. Sodium metaperiodate (1.07 g, 5.00 mmol) was added to a solution of the above residue in MeOH (10 ml) and H_2O (5 ml) at room temperature. Then the reaction mixture was stirred for 40 min. The mixture was poured into ice/water and AcOEt, and the organic layer was washed with brine, dried over Na_2SO_4 and removed under reduced pressure. The residue was purified by silica gel column chromatography (20% AcOEt/*n*-hexane) to give **8** (501 mg, 93%) as a colorless crystalline solid, mp 122—127 °C. MS (FAB) *m/z*: 1078 (MH^+). $[\alpha]_D^{24}$ −21.6° (*c* = 1.0, $CHCl_3$). *Anal.* Calcd for $C_{57}H_{83}NO_{15}Si_2 \cdot H_2O$: C, 62.43; H, 7.81; N, 1.28. Found: C, 62.27; H, 7.82; N, 1.22. IR (KBr): 3450, 2958, 1757, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.31—0.48 (m, 6H), 0.54—0.61 (m, 6H), 0.79 (t, 9H, *J* = 7 Hz), 0.96 (t, 9H, *J* = 7 Hz), 1.25 (s, 3H), 1.26 (s, 3H), 1.31 (s, 9H), 1.70 (s, 3H), 1.91 (s, 3H), 2.15—2.22 (m, 1H), 2.34—2.40 (m, 1H), 2.45—2.53 (m, 1H), 2.53 (s, 3H), 3.48 (s, 1H), 3.81 (d, 1H, *J* = 7 Hz), 4.18 (s, 1H), 4.19 (d, 1H, *J* = 8 Hz), 4.32 (d, 1H, *J* = 8 Hz), 4.44 (dd, 1H, *J* = 10, 7 Hz), 4.56 (s, 1H), 4.95 (d, 1H, *J* = 8 Hz), 5.12 (s, 1H), 5.30 (br, 1H), 5.47 (br, 1H), 5.68 (d, 1H, *J* = 7 Hz), 6.31 (t, 1H, *J* = 8 Hz), 7.29—7.39 (m, 3H), 7.37 (t, 2H, *J* = 8 Hz), 7.48 (t, 2H, *J* = 8 Hz), 7.59 (t, 1H, *J* = 8 Hz), 8.12 (d, 2H, *J* = 8 Hz), 9.85 (s, 1H).

13-*O*-[(2*R*,3*S*)-3-(*tert*-Butoxycarbonylamino)-2-hydroxy-3-phenylpropionyl]-10-deacetyl-10-*O*-(2-morpholinoethyl)baccatin III (9) Sodium cyanoborohydride (57 mg, 0.91 mmol) was added to a solution of **8** (98.5 mg, 0.0913 mmol), morpholine (0.081 ml, 0.91 mmol), and acetic acid (0.052 ml, 0.91 mmol) in EtOH (8 ml) at room temperature. After 1 h, the mixture was diluted with $CHCl_3$, and washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was then removed under reduced pressure. Pyridine (0.20 ml) and 48% aqueous hydrofluoric acid (0.60 ml) were added to a solution of the above residue in CH_3CN (4 ml) at 0 °C. Then the reaction mixture was stirred for 15 h at room temperature, and neutralized with $NaHCO_3$ under ice cooling. The mixture was extracted with AcOEt, and the organic layer was washed with brine, dried over Na_2SO_4 and removed under reduced pressure. The residue was purified by preparative TLC (20% MeOH/$CHCl_3$) and lyophilized with 1,4-dioxane to give **9** (52.1 mg, 62%) as an amorphous foam. IR (KBr): 3456, 3068, 2976, 2940, 1986, 1722, 1606, 1586 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (s, 3H), 1.20 (s, 3H), 1.34 (s, 9H), 1.67 (s, 3H), 1.78—1.85 (m, 1H), 1.88 (s, 3H), 2.27 (m, 2H), 2.36 (s, 3H), 2.51—2.68 (m, 7H), 3.63—3.80 (m, 6H), 3.84 (d, 1H, *J* = 7 Hz), 4.12 (d, 1H, *J* = 8 Hz), 4.22 (dd, 1H, *J* = 10, 7 Hz), 4.29 (d, 1H, *J* = 8 Hz), 4.62 (s, 1H), 4.94 (d, 1H, *J* = 8 Hz), 5.09 (s, 1H), 5.27 (d, 1H, *J* = 10 Hz), 5.44 (br, 1H), 5.66 (d, 1H, *J* = 7 Hz), 6.21 (t, 1H, *J* = 8 Hz), 7.29—7.42 (m, 5H), 7.49 (t, 2H, *J* = 8 Hz), 7.61 (t, 1H, *J* = 8 Hz), 8.09 (d, 2H, *J* = 8 Hz).

13-*O*-[(2*R*,3*S*)-3-(*tert*-Butoxycarbonylamino)-2-hydroxy-3-phenylpropionyl]-10-deacetyl-10-*O*-(2-thiomorpholinoethyl)baccatin III (10) Following the procedure described for **9**, compound **8** was converted to **10** (42 mg, 61%) by using thiomorpholine as a *sec*-amine. **10**: amorphous foam. IR (KBr): 3456, 2980, 2944, 1722 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (s, 3H), 1.21 (s, 3H), 1.35 (s, 9H), 1.68 (s, 3H), 1.85—1.92 (m, 1H), 1.88 (s, 3H), 2.22—2.27 (m, 2H), 2.37 (s, 3H), 2.52—2.58 (m, 1H), 2.67—2.73 (m, 6H), 2.82—2.90 (m, 4H), 3.63—3.66 (m, 1H), 3.75—3.78 (m, 1H), 3.85 (d, 1H, *J* = 7 Hz), 4.17 (d, 1H, *J* = 8 Hz), 4.22 (dd, 1H, *J* = 10, 7 Hz), 4.30 (d, 1H, *J* = 8 Hz), 4.62 (s, 1H), 4.94 (d, 1H, *J* = 8 Hz), 5.06 (s, 1H), 5.25 (br, 1H), 5.39 (d, 1H, *J* = 10 Hz), 5.66 (d, 1H, *J* = 7 Hz), 6.22 (t, 1H, *J* = 8 Hz), 7.31—7.42 (m, 5H), 7.49 (t, 2H, *J* = 8 Hz), 7.61 (t, 1H, *J* = 8 Hz), 8.10 (d, 2H, *J* = 8 Hz).

13-*O*-[(2*R*,3*S*)-3-(*tert*-Butoxycarbonylamino)-2-hydroxy-3-phenylpropionyl]-10-deacetyl-10-*O*-(2-piperidinoethyl)baccatin III (11) Following the procedure described for **9**, compound **8** was converted to **11** (17.5 mg, 26%) by using piperidine as a *sec*-amine. **11**: amorphous foam. IR (KBr): 3440, 2940, 1722 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (s, 3H), 1.20 (s, 3H), 1.34 (s, 9H), 1.46 (br, 2H), 1.67 (s, 3H), 1.85—1.89 (m, 1H), 1.90 (s, 3H), 2.23—2.28 (m, 2H), 2.36 (s, 3H), 2.45—2.73 (m, 7H), 3.74—3.84 (m, 3H), 4.17 (d, 1H, *J* = 8 Hz), 4.25 (dd, 1H, *J* = 10, 7 Hz), 4.29 (d, 1H, *J* = 8 Hz), 4.62 (s, 1H), 4.94 (d, 1H, *J* = 8 Hz), 5.20 (s, 1H), 5.25 (br, 1H), 5.45 (br, 1H), 5.66 (d, 1H, *J* = 7 Hz), 6.21 (t, 1H, *J* = 8 Hz), 7.31—7.39 (m, 5H), 7.49 (t, 2H, *J* = 8 Hz), 7.60 (t, 1H, *J* = 8 Hz), 8.10 (d, 2H, *J* = 8Hz).

13-*O*-[(2*R*,3*S*)-3-(*tert*-Butoxycarbonylamino)-2-hydroxy-3-phenylpropionyl]-10-deacetyl-10-*O*-[2-(1-pyrrolidinyl)ethyl]baccatin III (12) Following the procedure described for **9**, compound **8** was converted to **12** (19.5 mg, 29%) by using pyrrolidine as a *sec*-amine. **12**: amorphous foam. IR (KBr): 3448, 2976, 2820, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.21 (s, 6H), 1.34 (s, 9H), 1.67 (s, 3H), 1.80 (br, 4H), 1.90 (s, 3H), 2.23—2.28 (m, 2H), 2.36 (s, 3H), 2.45—2.53 (m, 1H), 2.70 (br, 4H), 2.85 (br, 2H), 3.74—3.81 (m, 2H), 3.83 (d, 1H, *J* = 7 Hz), 4.17 (d, 1H, *J* = 8 Hz), 4.24 (dd, 1H, *J* = 10, 7 Hz), 4.29 (d, 1H, *J* = 8 Hz), 4.61 (s, 1H), 4.94 (d, 1H, *J* = 8 Hz), 5.20 (s, 1H), 5.25 (br, 1H), 5.45 (br, 1H), 5.66 (d, 1H, *J* = 7 Hz), 6.21 (t, 1H, *J* = 8 Hz), 7.31—7.40 (m, 5H), 7.49 (t, 2H, *J* = 8 Hz), 7.60 (t, 1H, *J* = 8 Hz), 8.10 (d, 2H, *J* = 8 Hz).

10-*O*Allyl-13-*O*-[(2*R*,3*S*)-3-(*tert*-butoxycarbonylamino)-3-phenyl-2-(triethylsilyloxy)propionyl]-4,10-dideacetyl-4-*O*-propionyl-7-*O*-triethylsilylbaccatin III (13) Following the procedure described for **7**, compound **5b** was converted to **13** (65.6 mg, 83%). **13**: colorless solid, mp 101—106 °C. MS (FAB) *m/z*: 1090 (MH^+). $[\alpha]_D^{24}$ −17.6° (*c* = 0.84, $CHCl_3$). *Anal.* Calcd for $C_{59}H_{87}NO_{14}Si_2$: C, 64.98; H, 8.04; N, 1.28. Found: C, 64.81; H, 8.22; N, 1.25. IR (KBr): 3454, 2958, 1756, 1718 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.33—0.49 (m, 6H), 0.52—0.62 (m, 6H), 0.79 (t, 9H, *J* = 8 Hz), 0.96 (t, 9H, *J* = 8 Hz), 1.24 (s, 6H), 1.34 (s, 9H), 1.38 (t, 3H, *J* = 7 Hz), 1.69 (s, 3H), 1.90 (s, 3H), 1.88—1.92 (m, 1H), 2.18—2.27 (m, 1H), 2.33—2.41 (m, 1H), 2.45—2.52 (m, 1H), 2.73—2.85 (m, 2H), 3.83 (d, 1H, *J* = 7 Hz), 4.05 (q, 2H, *J* = 8 Hz), 4.20 (d, 1H, *J* = 8 Hz), 4.33 (d, 1H, *J* = 8 Hz), 4.43 (dd, 1H, *J* = 10, 7 Hz), 4.54 (s, 1H), 4.90 (d, 1H, *J* = 8 Hz), 4.99 (s, 1H), 5.20 (dd, 1H, *J* = 10, 1.5 Hz), 5.23 (d, 1H, *J* = 10 Hz), 5.32 (dd, 1H, *J* = 17, 1.5 Hz), 5.47(d, 1H, *J* = 10 Hz), 5.69 (d, 1H, *J* = 7 Hz), 5.94—6.02 (m, 1H), 6.26 (t, 1H, *J* = 8 Hz), 7.28—7.40 (m, 5H), 7.48 (t, 2H, *J* = 8 Hz), 7.59 (t, 1H, *J* = 8 Hz), 8.13 (d, 2H, *J* = 8 Hz).

13-*O*-[(2*R*,3*S*)-3-(*tert*-Butoxycarbonylamino)-2-hydroxy-3-phenylpropionyl]-4,10-dideacetyl-4-*O*-propionyl-10-*O*-(2-thiomorpholinoethyl)-baccatin III (14) Following the procedure described for **8** and **10**, compound **13** was converted to **14** (20.4 mg, 36%). **14**: amorphous foam. IR (KBr): 3456, 2980, 2944, 1722 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (s, 3H), 1.21 (t, 3H, *J* = 7 Hz), 1.22 (s, 3H), 1.34 (s, 9H), 1.67 (s, 3H), 1.76—1.88 (m, 1H), 1.89 (s, 3H), 2.29 (m, 2H), 2.53—2.59 (m, 3H), 2.64—2.78 (m, 6H), 2.79—2.89 (m, 4H), 3.62—3.67 (m, 1H), 3.71—3.85 (m, 1H), 3.84 (d, 1H, *J* = 7 Hz), 4.17 (d, 1H, *J* = 8 Hz), 4.26 (dd, 1H, *J* = 10, 7 Hz), 4.31 (d, 1H, *J* = 8 Hz), 4.62 (s, 1H), 4.89 (d, 1H, *J* = 8 Hz), 5.05 (s, 1H), 5.23 (br, 1H), 5.32 (d, 1H, *J* = 9 Hz), 5.67 (d, 1H, *J* = 7 Hz), 6.21 (t, 1H, *J* = 8 Hz), 7.31—7.42 (m, 5H), 7.50 (t, 2H, *J* = 8 Hz), 7.62 (t, 1H, *J* = 8 Hz), 8.12 (d, 2H, *J* = 8Hz).

10-*O*-Allyl-13-*O*-[(2*R*,3*R*)-3-(*tert*-butoxycarbonylamino)-3-(2-furyl)-2-(triisopropylsilyloxy)propionyl]-4,10-dideacetyl-4-*O*-propionyl-7-*O*-triethylsilylbaccatin III (16) Following the procedure described for **7**, compound **5b** was converted to **16** (67.8 mg, 86%) by using the *β*-lactam (**15**). **16**: colorless crystalline solid, mp 96—99 °C. MS (FAB) *m/z*: 1122 (MH^+). $[\alpha]_D^{22}$ −32.8° (*c* = 0.92, $CHCl_3$). *Anal.* Calcd for $C_{60}H_{91}NO_{15}Si_2$: C, 64.20; H, 8.17; N, 1.25. Found: C, 64.01; H, 8.36; N, 1.12. IR (KBr): 3457, 2950, 1760, 1718 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.52—0.62 (m, 6H), 0.94—1.01 (m, 30H), 1.21 (s, 3H), 1.24 (s, 3H), 1.35 (s, 9H), 1.37 (t, 3H, *J* = 7 Hz), 1.69 (s, 3H), 1.92 (s, 3H), 1.87—1.94 (m, 1H), 2.31—2.36 (m, 2H), 2.46—2.56 (m, 1H), 2.71—2.79 (m, 2H), 3.84 (d, 1H, *J* = 7 Hz), 4.00—4.11 (m, 2H), 4.19 (d, 1H, *J* = 8 Hz), 4.31 (d, 1H, *J* = 8 Hz), 4.42 (dd, 1H, *J* = 10, 7 Hz), 4.89 (d, 1H, *J* = 8 Hz), 4.99 (s, 1H), 5.20 (d, 1H, *J* = 11 Hz), 5.24—5.34 (m, 3H), 5.68 (d, 1H, *J* = 7 Hz), 5.95—6.02 (m, 1H), 6.21 (t, 1H, *J* = 8 Hz), 6.27 (d, 1H, *J* = 3.5 Hz), 6.37 (dd, 1H, *J* = 3.5, 2 Hz), 7.39 (s, 1H), 7.46 (t, 2H, *J* = 8 Hz), 7.57 (t, 1H, *J* = 8 Hz), 8.11 (d, 2H, *J* = 8 Hz).

13-*O*-[(2*R*,3*R*)-3-(*tert*-Butoxycarbonylamino)-3-(2-furyl)-2-(triisopropylsilyloxy)propionyl]-4,10-dideacetyl-10-*O*-(formylmethyl)-4-*O*-propionyl-7-*O*-triethylsilylbaccatin III (17) Following the procedure described for **8**, compound **16** was converted to **17** (55.0 mg, 81%). **17**: colorless crystalline solid, mp 111—118 °C. MS (FAB) *m/z*: 1124 (MH^+). $[\alpha]_D^{22}$ −31.6° (*c* = 0.95, $CHCl_3$). *Anal.* Calcd for $C_{59}H_{89}NO_{16}Si_2 \cdot 3/2H_2O$: C, 61.54; H, 8.05; N, 1.22. Found: C, 61.68; H, 8.13; N, 1.21. IR (KBr): 3458, 2950, 1759, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.53—0.63 (m, 6H), 0.94—1.01 (m, 30H), 1.22 (s, 3H), 1.25 (s, 3H), 1.34 (s, 9H), 1.36 (t, 3H, *J* = 7 Hz), 1.69 (s, 3H), 1.92 (s, 3H), 2.32—2.36 (m, 2H), 2.46—2.53 (m, 1H), 2.69—2.78 (m, 2H), 3.47 (s, 1H), 3.80 (d, 1H, *J* = 7 Hz), 4.16 (s, 1H), 4.18 (d, 1H, *J* = 8 Hz), 4.31 (d, 1H, *J* = 8 Hz), 4.43 (dd, 1H, *J* = 10, 7 Hz), 4.90 (d, 1H, *J* = 8 Hz), 4.98 (s, 1H), 5.13 (s, 1H), 5.24—5.30 (m, 2H), 5.68 (d, 1H, *J* = 7 Hz), 6.20 (t, 1H, *J* = 8 Hz), 6.27 (d, 1H, *J* = 3.5 Hz), 6.37 (dd, 1H, *J* = 3.5, 2 Hz), 7.39 (s, 1H), 7.47 (t, 2H, *J* = 8 Hz), 7.57 (t, 1H, *J* = 8Hz), 8.11 (d, 2H, *J* = 8Hz), 9.85 (s, 1H).

13-*O*-[(2*R*,3*R*)-3-(*tert*-Butoxycarbonylamino)-3-(2-furyl)-2-hydroxy-

propionyl]-4,10-dideacetyl-10-*O*-(2-morpholinoethyl)-4-*O*-propionylbaccatin III (18) Following the procedure described for **9**, compound **17** was converted to **18** (27.6 mg, 61%) by using morpholine as a *sec*-amine. **18**: amorphous foam. IR (KBr): 3464, 2980, 2944, 1722 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (s, 3H), 1.21 (s, 3H), 1.29 (t, 3H, J=7 Hz), 1.34 (s, 9H), 1.67 (s, 3H), 1.78—1.85 (m, 1H), 1.93 (s, 3H), 2.26—2.40 (m, 2H), 2.52—2.73 (m, 9H), 3.64—3.80 (m, 6H), 3.86 (d, 1H, J=7 Hz), 4.18 (d, 1H, J=8 Hz), 4.27 (dd, 1H, J=10, 7 Hz), 4.31 (d, 1H, J=8 Hz), 4.70 (s, 1H), 4.91 (d, 1H, J=8 Hz), 5.09 (s, 1H), 5.23 (d, 1H, J=10 Hz), 5.31 (d, 1H, J=10 Hz), 5.68 (d, 1H, J=7 Hz), 6.23 (t, 1H, J=8 Hz), 6.34 (d, 1H, J=3.5 Hz), 6.39 (dd, 1H, J=3.5, 2 Hz), 7.43 (s, 1H), 7.48 (t, 2H, J=8 Hz), 7.60 (t, 1H, J=8 Hz), 8.12 (d, 2H, J=8 Hz).

13-*O*-[(2*R*,3*R*)-3-(*tert*-Butoxycarbonylamino)-3-(2-furyl)-2-hydroxypropionyl]-4,10-dideacetyl-4-*O*-propionyl-10-*O*-(2-thiomorpholinoethyl)-baccatin III (19) Following the procedure described for **9**, compound **17** was converted to **19** (26.5 mg, 62%) by using thiomorpholine as a *sec*-amine. **19**: amorphous foam. IR (KBr): 3456, 2980, 2944, 1722 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (s, 3H), 1.21 (s, 3H), 1.29 (t, 3H, J=7 Hz), 1.34 (s, 9H), 1.67 (s, 3H), 1.78—1.85 (m, 1H), 1.92 (s, 3H), 2.25—2.40 (m, 2H), 2.53—2.61 (m, 1H), 2.66—2.72 (m, 8H), 2.78—2.90 (m, 4H), 3.61—3.67 (m, 1H), 3.72—3.78 (m, 1H), 3.86 (d, 1H, J=7 Hz), 4.17 (d, 1H, J=8 Hz), 4.27 (dd, 1H, J=10, 7 Hz), 4.31 (d, 1H, J=8 Hz), 4.71 (s, 1H), 4.81 (d, 1H, J=8 Hz), 5.05 (s, 1H), 5.22 (d, 1H, J=10 Hz), 5.31 (d, 1H, J=10 Hz), 5.68 (d, 1H, J=7 Hz), 6.23 (t, 1H, J=8 Hz), 6.34 (d, 1H, J=3.5 Hz), 6.39 (dd, 1H, J=3.5, 2 Hz), 7.43 (s, 1H), 7.48 (t, 2H, J=8 Hz), 7.60 (t, 1H, J=8 Hz), 8.12 (d, 2H, J=8 Hz).

Methanesulfonic Acid Salt (**18a**): Methanesulfonic acid (3.1 ml of a 2 mg/ml aqueous solution, 0.065 mmol) was added to a solution of **18** (59 mg, 0.064 mmol) in *tert*-butyl alcohol (2 ml) and water (1.5 ml) cooled to 0—5 °C, and the mixture was stirred for 2 min and filtered through a Millipore filter (0.2 μm). The filtrate was freeze-dried to give the methanesulfonic acid salt (**18a**) (51 mg, 78%) as an amorphous foam, mp 157—161 °C (dec.). MS (FAB) m/z: 925 (MH^+). *Anal.* Calcd for $C_{49}H_{68}N_2O_{19}S \cdot 2H_2O$: C, 55.67; H, 6.86; N, 2.65; S, 3.03. Found: C, 55.76; H, 6.91; N, 2.55; S, 3.23. IR (KBr): 3432, 2968, 2940, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.17 (s, 3H), 1.20 (s, 3H), 1.27 (t, 3H, J=7 Hz), 1.34 (s, 9H), 1.67 (s, 3H), 1.85—1.92 (m, 1H), 2.04 (s, 3H), 2.21—2.40 (m, 2H), 2.45—2.56 (m, 1H), 2.65—2.70 (m, 2H), 2.78 (s, 3H), 3.04 (m, 4H), 3.29 (br, 1H), 3.81 (d, 1H, J=7 Hz), 3.92—4.10 (m, 6H), 4.16 (d, 1H, J=8 Hz), 4.30 (d, 1H, J=8 Hz), 4.35 (dd, 1H, J=10, 7 Hz), 4.70 (s, 1H), 4.91 (d, 1H, J=8 Hz), 5.25—5.30 (m, 2H), 5.54 (s, 1H), 5.67 (d, 1H, J=7 Hz), 6.21 (t, 1H, J=8 Hz), 6.34 (d, 1H, J=3.5 Hz), 6.37 (dd, 1H, J=3.5, 2 Hz), 7.43 (s, 1H), 7.48 (t, 2H, J=8 Hz), 7.60 (t, 1H, J=8 Hz), 8.12 (d, 2H, J=8 Hz).

Methanesulfonic Acid Salt (**19a**): Following the procedure described above, compound **19** was converted to the methanesulfonic acid salt (**19a**) (28 mg, 85%). **19a**: amorphous foam, mp 163—168 °C (dec.). MS (FAB) m/z: 941 (MH^+). *Anal.* Calcd for $C_{49}H_{68}N_2O_{18}S_2 \cdot 2H_2O$: C, 54.84; H, 6.76; N, 2.61; S, 5.97. Found: C, 54.99; H, 6.71; N, 2.46; S, 5.75. IR (KBr): 3444, 2984, 1718 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.17 (s, 3H), 1.20 (s, 3H), 1.27 (t, 3H, J=7 Hz), 1.34 (s, 9H), 1.68 (s, 3H), 1.88 (m, 1H), 2.04 (s, 3H), 2.23—2.39 (m, 2H), 2.48—2.56 (m, 1H), 2.65—2.70 (m, 2H), 2.80 (s, 3H), 3.10—3.48 (m, 10H), 3.81 (d, 1H, J=7 Hz), 3.85—3.95 (m, 2H), 4.16 (d, 1H, J=8 Hz), 4.30 (d, 1H, J=8 Hz), 4.36 (dd, 1H, J=10, 7 Hz), 4.70 (s, 1H), 4.90—4.95 (m, 2H), 5.25 (d, 1H, J=10 Hz), 5.30 (d, 1H, J=10 Hz), 5.67 (d, 1H, J=7 Hz), 6.22 (t, 1H, J=8 Hz), 6.34 (s, 1H), 6.38 (s, 1H), 7.43 (s, 1H), 7.48 (t, 2H, J=8 Hz), 7.60 (t, 1H, J=8 Hz), 8.12 (d, 2H, J=8 Hz).

10-*O*-Allyl-13-*O*-[(2*R*,3*R*)-3-(*tert*-butoxycarbonylamino)-3-(2-furyl)-2-(triisopropylsilyloxy)propionyl]-4-*O*-butyryl-4,10-dideacetyl-7-*O*-triethylsilylbaccatin III (20) Following the procedure described for **7**, compound **5c** was converted to **20** (72.9 mg, 93%) by using the β-lactam (**15**). **20**: colorless crystalline solid, mp 86—92 °C. MS (FAB) m/z: 1136 (MH^+). $[\alpha]_D^{22}$ −30.5° (c=0.72, $CHCl_3$). *Anal.* Calcd for $C_{61}H_{93}NO_{15}Si_2$: C, 64.46; H, 8.25; N, 1.23. Found: C, 64.41; H, 8.20; N, 1.23. IR (KBr): 3455, 2953, 1760, 1718 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.52—0.63 (m, 6H), 0.94—1.01 (m, 30H), 1.05 (t, 3H, J=7 Hz), 1.20 (s, 3H), 1.24 (s, 3H), 1.35 (s, 9H), 1.69 (s, 3H), 1.92 (s, 3H), 1.83—1.95 (m, 1H), 2.25—2.36 (m, 2H), 2.46—2.56 (m, 1H), 2.58—2.63 (m, 2H), 2.76—2.80 (m, 1H), 3.85 (d, 1H, J=7 Hz), 3.99—4.11 (m, 2H), 4.18 (d, 1H, J=8 Hz), 4.29 (d, 1H, J=8 Hz), 4.41 (dd, 1H, J=10, 7 Hz), 4.89 (d, 1H, J=8 Hz), 5.00 (s, 1H), 5.18—5.34 (m, 4H), 5.67 (d, 1H, J=7 Hz), 5.94—6.02 (m, 1H), 6.20 (t, 1H, J=8 Hz), 6.27 (d, 1H, J=3.5 Hz), 6.37 (dd, 1H, J=3.5, 2 Hz), 7.37 (s, 1H), 7.46 (t, 2H, J=8 Hz), 7.57 (t, 1H, J=8 Hz), 8.11 (d, 2H, J=8 Hz).

13-*O*-[(2*R*,3*R*)-3-(*tert*-Butoxycarbonylamino)-3-(2-furyl)-2-hydroxypropionyl]-4-*O*-butyryl-4,10-dideacetyl-10-*O*-(2-morpholinoethyl)baccatin III (21) Following the procedure described for **8** and **9**, compound **20** was converted to **21** (24.2 mg, 40%). **21**: amorphous foam. IR (KBr): 3456, 2972, 2944, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.99 (t, 3H, J=7 Hz), 1.19 (s, 3H), 1.21 (s, 3H), 1.35 (s, 9H), 1.68 (s, 3H), 1.78—1.84 (m, 3H), 1.93 (s, 3H), 2.30—2.38 (m, 2H), 2.53—2.68 (m, 9H), 3.65—3.78 (m, 6H), 3.88 (d, 1H, J=7 Hz), 4.18 (d, 1H, J=8 Hz), 4.26 (dd, 1H, J=10, 7 Hz), 4.30 (d, 1H, J=8 Hz), 4.70 (s, 1H), 4.90 (d, 1H, J=8 Hz), 5.10 (s, 1H), 5.22 (d, 1H, J=10 Hz), 5.31 (d, 1H, J=10 Hz), 5.67 (d, 1H, J=7 Hz), 6.20 (t, 1H, J=8 Hz), 6.35 (d, 1H, J=3.5 Hz), 6.38 (dd, 1H, J=3.5, 2 Hz), 7.42 (s, 1H), 7.49 (t, 2H, J=8 Hz), 7.60 (t, 1H, J=8 Hz), 8.11 (d, 2H, J=8 Hz).

10-*O*-Allyl-13-*O*-[(3*S*)-3-(*tert*-butoxycarbonylamino)-2,2-difluoro-3-(2-furyl)propionyl]-4,10-dideacetyl-4-*O*-propionyl-7-*O*-triethylsilylbaccatin III (23) A solution of **22** (470 mg, 1.60 mmol) in toluene (5 ml) was added dropwise to a stirred solution of **5b** (190 mg, 0.266 mmol), dipyridyl carbonate (350 mg, 1.60 mmol), and 4-(dimethylamino)pyridine (32.5 mg, 0.266 mmol) in toluene (5 ml) at room temperature. The mixture was heated to 80 °C and stirred for 20 h. It was then cooled, diluted with AcOEt, washed with aqueous 1 N HCl, water, saturated aqueous sodium bicarbonate and brine, and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (5% acetone/$CHCl_3$) to give **23** (225mg, 86%) as a colorless crystalline solid, mp 102—107 °C. MS (FAB) m/z: 986 (MH^+). $[\alpha]_D^{23}$ −16.9° (c=1.0, $CHCl_3$). *Anal.* Calcd for $C_{51}H_{69}F_2NO_{14}Si$: C, 62.11; H, 7.05; N, 1.42. Found: C, 62.34; H, 7.07; N, 1.38. IR (KBr): 3451, 2977, 2958, 1770, 1722 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.51—0.64 (m, 6H), 0.95 (t, 9H, J=8 Hz), 1.21 (s, 3H), 1.24 (s, 3H), 1.31 (t, 3H, J=7 Hz), 1.42 (s, 9H), 1.68 (s, 3H), 1.87 (s, 3H), 1.85—1.92 (m, 1H), 2.25—2.27 (m, 2H), 2.45—2.53 (m, 1H), 2.59—2.61 (m, 2H), 3.79 (d, 1H, J=7 Hz), 3.99—4.07 (m, 2H), 4.15 (d, 1H, J=8 Hz), 4.30 (d, 1H, J=8 Hz), 4.41 (dd, 1H, J=10, 7 Hz), 4.87 (d, 1H, J=8 Hz), 4.96 (s, 1H), 5.19 (dd, 1H, J=10, 1.5 Hz), 5.29 (dd, 1H, J=17, 1.5 Hz), 5.38 (d, 1H, J=10 Hz), 5.62 (m, 1H), 5.66 (d, 1H, J=7 Hz), 5.92—6.02 (m, 1H), 6.27 (t, 1H, J=8 Hz), 6.40—6.44 (m, 2H), 7.45—7.50 (m, 3H), 7.61 (t, 1H, J=8 Hz), 8.11 (d, 2H, J=8 Hz).

13-*O*-[(3*S*)-3-(*tert*-Butoxycarbonylamino)-2,2-difluoro-3-(2-furyl)propionyl]-4,10-dideacetyl-4-*O*-propionyl-10-*O*-(2-thiomorpholinoethyl)baccatin III (24) Following the procedure described for **8** and **9**, compound **23** was converted to **24** (21.0 mg, 38%). **24**: amorphous foam. IR (KBr): 3464, 2984, 2944, 1768, 1724 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (s, 3H), 1.21 (s, 3H), 1.29 (t, 3H, J=7 Hz), 1.41 (s, 9H), 1.67 (s, 3H), 1.87 (s, 3H), 1.81—1.84 (m, 1H), 2.21—2.32 (m, 2H), 2.54—2.62 (m, 3H), 2.66—2.71 (m, 8H), 2.78—2.90 (m, 4H), 3.60—3.65 (m, 1H), 3.71—3.79 (m, 1H), 3.84 (d, 1H, J=7 Hz), 4.13 (d, 1H, J=8 Hz), 4.31 (d, 1H, J=8 Hz), 4.28 (dd, 1H, J=10, 7 Hz), 4.89 (d, 1H, J=8 Hz), 5.02 (s, 1H), 5.36 (d, 1H, J=10 Hz), 5.57—5.63 (m, 1H), 5.67 (d, 1H, J=7 Hz), 6.25 (t, 1H, J=8 Hz), 6.40—6.43 (m, 2H), 7.45—7.50 (m, 3H), 7.61 (t, 1H, J=8 Hz), 8.10 (d, 2H, J=8 Hz)

***In Vitro* Cytotoxicity** Growth inhibition experiments were carried out in 96-well microplates, and the amount of viable cells at the end of the incubation was determined by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[15] Thus, 500—5000 cells/well in 150 μl of medium were plated and grown for 24 h (P388 for 2 h), a drug (in 50 μl medium/well), or the medium alone as a control, was added, and cells were grown for an additional 3 d. After addition of MTT (20 μl, 20 mg/ml in phosphate-buffered saline), the medium was removed and the blue dye formed was dissolved in 150 μl of DMSO. The absorbance was measured at 540 nm using a Microplate Reader model 3550 (Bio-Rad Laboratories, Richmond, California, U.S.A.).

Preparation of Tubulin Tubulin was purified from porcine brain extracts by two cycles of polymerization and depolymerization, as previously described by Shelanski *et al.*[16] Brain tissue was homogenized at 4 °C with 5 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), pH 6.5, 0.5 mM $MgSO_4$, 1 mM ethylenebis(oxyethylenenitrilo)-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA), 50 mM KCl, 1 mM ATP and phenylmethylsulfonyl fluoride (PMSF) (1 mg per brain). The homogenate was then centrifuged at 26000 × ***g*** for 30 min at 4 °C. The supernatant was decanted and the residue was centrifuged at 50000 × ***g*** for 30 min at 4 °C. Again, the supernatant was decanted and the residue was mixed with an equal volume of 5 mM MES pH 6.5, 0.5 mM $MgSO_4$, 1 mM EGTA, 50 mM KCl, 1 mM ATP and 8 mM glycerol. The mixture was incubated at 37 °C for

40 min to allow assembly of microtubules and then centrifuged at 105000 × *g* for 45 min at 25 °C. The pellets were resuspended in 5 mM MES pH 6.5, 0.5 mM $MgSO_4$, 1 mM EGTA, 50 mM KCl, 1 mM guanosine 5′-triphosphate (GTP), trisodium salt and incubated at 4 °C for 30 min to permit disassembly of microtubules. After centrifugation at 105000 × *g* for 60 min at 4 °C, the supernatant was decanted and mixed with an equal volume of 5 mM MES pH 6.5, 0.5 mM $MgSO_4$, 1 mM EGTA, 50 mM KCl, 1 mM ATP and 8 mM glycerol (1 cycle microtubule proteins, 1-cycle MTS).

Microtubule Disassembly Assay Immediately before use, a stock solution of 1-cycle MTS was carried through an additional cycle of polymerization and depolymerization to give 2-cycle MTS. The 2-cycle MTS was adjusted to a concentration of 5 mg/ml in 5 mM MES pH 6.5, 0.5 mM $MgSO_4$, 1 mM EGTA, 50 mM KCl, 1 mM GTP. A taxoid in DMSO was added to the 2-cycle MTS solution in a 96-well plate. The plate was incubated at 37 °C for 40 min to allow assembly of microtubules. The polymerization was evaluated by measuring turbidity at 405 nm. Then the temperature was shifted to 4 °C, and 20 min later the cold-reversibility was also evaluated. The depolymerization rate was calculated from the difference in turbidity between before and after the cold treatment. The IC_{50} value indicates the concentration of a taxoid with which the rate is decreased by 50%.

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