Structure−Activity Relationships of Ring C-Secotaxoids. 1. Acylative Modifications†

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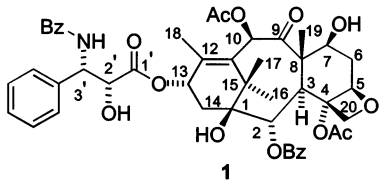
The acylative modification of IDN 5390 (**3a**), a 7,8-secotaxoid under preclinical development, was investigated. A modest decrease of potency was observed upon acylation of the primary and the enolic hydroxyls, suggesting that, just like in paclitaxel, the hydroxyl groups in the upper right-hand sector are not critical for cytotoxicity. The activity of these analogues, and especially of the chemically robust carbonates **3c** and **3d**, makes it unlikely that the activity of IDN 5390 is due to in vivo oxidation to a fledgling 7-aldehyde and re-aldolization to the corresponding taxane derivative.

Over the past 15 years, the structure−activity relationships of paclitaxel (Taxol, **1**) have been thoroughly investigated, identifying the major sites involved in its tubulin binding and highlighting the relevance of the baccatin core to significant antitumor activity.[1] Despite the propensity of the taxane skeleton to undergo skeletal rearrangements, the constitutional modifications of the baccatin core of **1** have proved generally disappointing in terms of the resultant biological activity. Within the few exceptions discovered so far, two are centered on C-7 and involve the closure of a further ring between C-7 and C-19 (7,19-cyclotaxoid **2**)[2] and the cleavage of the 7,8-bond (7,8-secotaxoid **3a**, IDN 5390),[3] respectively. While 7,19-cyclotaxanes do not deviate significantly from the inverted-cup geometry of baccatin, the paclitaxel mimicry of **3a** is surprising, since the cleavage of the 7,8-bond has a profound conformational effect, leading to a flexible system that adopts more than one conformation at room temperature.[4] Compound **3a** substantially overlaps with paclitaxel in terms of the cytotoxicity pattern in the NCI 60-cell line panel and tubulin binding.[5] Yet, **3a** showed limited toxicity toward endothelial cells and an excellent tolerability upon systemic administration, over 1 order of magnitude better than **1**.[5] These properties, coupled to a remarkable antimotility activity, a good oral availability, and a potent anticancer activity in a variety of human tumor xenografts, combine to make **3a** an interesting antiangiogenic candidate for protracted schedules of anticancer treatments.[6]

The paclitaxel-like activity of **3a** is somewhat unexpected, since homologation[7] and contraction[8] of ring C were both detrimental to the activity of first-generation taxoids. On the other hand, the structure−activity relationships of paclitaxel have been investigated mainly with point mutations,[9] and little is known on the effect of combined changes, like those featured by **3a**. Compared to **1**, **3a** shows differences not only in the upper right-hand baccatin sector but also on the side chain, which belongs to the norstatin and not to the phenylisoserine series. The observation that the replacement of the norstatin chain of **3a** with the side chain of **1** leads to an over 50-fold decrease



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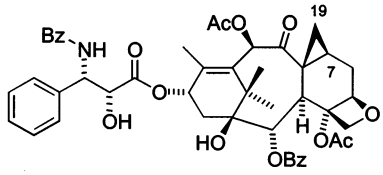

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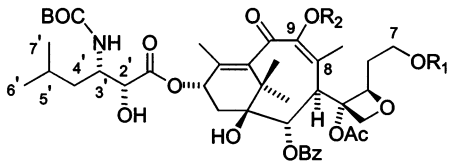
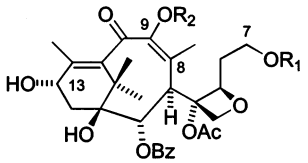


	R_1	R_2
3a	H	H
3b	Ac	Ac
3c	BOC	BOC
3d	H	Cbz
3e	H	Ac



	R_1	R_2
4a	H	H
4b	TES	TES
4c	Ac	Ac
4d	BOC	BOC
4e	CCl_3CO	CCl_3CO
4f	H	TES
4g	H	BOC
4h	H	Cbz
4i	Ac	H
4l	Piv	H
4m	Trityl	Cbz

† Dedicated to the late Dr. Monroe E. Wall and to Dr. Mansukh C. Wani of Research Triangle Institute for their pioneering work on bioactive natural products.

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of cytotoxicity dramatically highlights the relevance of combined changes on the terpenoid core and the amino acidic side chain of taxoids[3] and underlies the suggestion that the norstatin chain of the secotaxane **3a** binds to the paclitaxel binding cleft on tubulin in a different way than the amino acid chains of cytotoxic taxanes.[10]

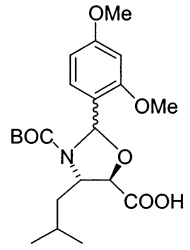
Structure−activity investigations on ring C-secotaxanes have so far focused only on the side chain,[3] and nothing is known on the effect of modifications on the terpenoid core. The study of these compounds has long been hampered by the limited availability of the key intermediate C-secobaccatin **4a**.[4] Following the development of an expeditious entry into **4a** and related compounds,[11] we have embarked into a systematic structure−activity study on ring C-secotaxoids, starting with the acylative modification of the primary and the enolic hydroxyls. Cytotoxicity against MCF-7 breast cancer cells was used as the end-point. While undoubtedly intrinsically reductive in terms of overall biologic activity, cytotoxicity is nevertheless an important facet of the anticancer profile of **3a** and an indispensable starting point for more advanced and focused mechanistic investigations.

Results and Discussion

The ring C-secobaccatin **4a** was prepared in two steps[11] from commercially available 10-deacetylbaccatin III and served as a starting material for the preparation of the acylated analogues of IDN 5390. Assuming the refractory nature of the tertiary bridgehead 1-hydroxyl, three hydroxyls of **4a** can be esterified, namely, the primary at C-7, the enolic at C-9, and the secondary at C-13. Despite a better structural diversity compared to the three secondary hydroxyls of 10-deacetylbaccatin III, the chemoselective hydroxyl manipulation of **4a** proved more difficult than expected. Thus, under nucleophilic catalysis and at complete conversion of the starting material, the reaction with triethylsilyl chloride (TES-Cl) and a series of acylating reagents such as anhydrides (Ac_2O, BOC_2O), acid chlorides (CCl_3COCl), and isoureas (from carboxylic acids and DCC) afforded mainly 7,9-diacylated (silylated) derivatives (**4b**−**e**). The 9-TES (**4f**) and 9-BOC derivative (**4g**) were obtained as byproducts from the reaction with TES-Cl and BOC_2O, respectively, an observation suggesting that the enolic 9-hydroxyl is more reactive than the primary 7-hydroxyl, but that the difference is generally modest. An exception was the reaction with benzyl chloroformate (Cbz-Cl), which gave the 9-derivative **4h** as the major reaction product in acceptable isolated yield (63%), while also the reaction with BOC_2O could be steered to the generation of the 9-mono-BOC derivative **4g** using stoichiometric amounts of the acylating agent and quenching the reaction at incomplete conversion.

The structure elucidation of ring C-secotaxoids is not trivial, since most proton signals are broad and unresolved at room temperature, with the resonances of some carbons remaining undetectable even at high temperatures (>100 °C).[4] Nevertheless, the chemoselectivity of the acylation reactions could be ascertained in a relatively simple way, since H-13 is clearly identifiable in the room-temperature ^{1}H NMR spectra of secotaxanes. Furthermore, modifications at the enolic hydroxyl are evident from the disappearance of the exchangeable signal of the enolic proton around δ 6.50 in $CDCl_3$, with the degree of derivatization being inferred from the molecular weight.

The 7,9-diacetyl (**4c**) and diBOC (**4d**) derivatives were further elaborated into **3b** and **3c**, respectively, by esterification with the protected norstatin side chain of ortataxel (**5**)[12] and deprotection of the crude reaction mixture with HCl in methanol. The 9-Cbz derivative **4h** was first protected at the primary hydroxyl as a trityl ether and then coupled with **5** and deprotected, under mild acidic conditions, at both the side chain and the 7-hydroxyl, eventually affording **3d**. The NMR spectra of the final products were better resolved as compared to the intermediate secobaccatins, and all proton resonances could be assigned at 60 °C, confirming the structure elucidation of the ring C-secobaccatin precursors.



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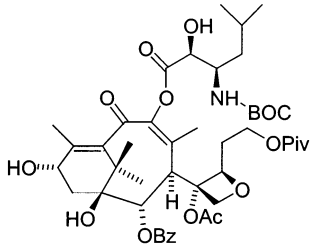

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The primary hydroxyl of **4a** could be chemoselectively esterified under the so-called Scheeren−Holton conditions, that is, by reaction with an acid chloride or anhydride and $CeCl_3$ in THF.[13] In both cases, the crude reaction mixture was a ca. 5:1 mixture of the 7- and 9-acyl derivatives, but the major 7-acyl derivative could be purified by crystallization (**4i**) or by chromatography (**4l**). The mechanistic basis for the remarkable chemoselectivity of the Scheeren−Holton acylation is unknown.[14] It is remarkable, however, that, just like in 10-deacetylbaccatin III, also with the secobaccatin **4a** the reactivity of the hydroxyls is different from that observed under nucleophilic conditions of acylation. Treatment of the 7-acetate **4i** with the protected amino acid **5** afforded, after deprotection, a major reaction product, identified as the 13-aminoacylester **3e**. Thus, esterification had occurred at C-13, but acyl migration from the primary to the enolic hydroxyl had also taken place, as evidenced by the upfield shift of the H-7α,β protons ($\Delta\delta$ ca. −0.20 and −0.40, respectively) and the chemical shift of the acetyl (δ 2.28), typical of an enol ester rather than an alkyl ester. A possible explanation for the outcome of the reaction is that a 7,9-semiorthoester is formed in the acylation conditions. This prevents the interaction of the reactive 9-hydroxyl with the side-chain carbonyl and then evolves into a 9-acetate during the aqueous workup. Surprisingly, treatment of the 7-pivalate **4l** with **5** afforded, after side-chain deprotection, the 9-ester **6** as the only reaction product, possibly because the bulky *tert*-butyl group of the pivaloyl moiety prevents interaction of the 7-acyl carbonyl with the 9-hydroxyl, leaving it free to attack the carbodiimide-activated side-chain carbonyl. Another remarkable observation regarding the reactivity of the 7- and 9-hydroxyl of secotaxanes is the possibility of chemoselectively desilylating the 7-hydroxyl of 7,9-diTES secoDAB

Table 1. Cytotoxicity [IC_{50} (nM))][a] of the Ring C-Secotaxanes **3a**−**e** and **6**

compound	MCF-7	MCF7-R
paclitaxel (**1**)	1.4 ± 0.3	354 ± 15
3a	14 ± 0.9	1639 ± 152
3b	36 ± 2.1	956 ± 49
3c	51 ± 3.9	736 ± 23
3d	53 ± 3.9	1205 ± 68
3e	18 ± 2.9	1000 ± 57
6	>3000	>3000

[a] Concentration of compound that inhibits 50% (IC_{50}, nM) of the growth of the human tumor cell line after 72 h drug exposure.

(**4b**) by treatment with $CeCl_3$ in THF.[15] This reaction affords an easy entry into the 9-protected derivative **4f**, a compound that could not be obtained in synthetically useful amounts by direct silylation of **4a**.

The cytotoxic effect of **3b**−**e** and **6** was investigated on MCF-7 breast cancer cells, a line where **3a** is ca. 10-fold less potent than paclitaxel (Table 1). While **6**, having the side chain transposed at the enolic hydroxyl, was inactive, acylation of the 9-hydroxyl (compounds **3d** and **3e**) caused only a modest decrease of activity, more evident in the 7,9-diacetate **3b** and the 7,9-diBOC derivative **3c**.

Taken together, these data show a substantial retention of the two-digit nanomolar cytotoxicity of the 9- and 7,9-acyl derivatives of IDN 5390 in MCF-7 cells, suggesting that, just like in paclitaxel, the hydroxyls in the right-hand upper sector are not critical elements of the pharmacophore[1] and are suitable sites for bioactivity modulation. By further analogy with paclitaxel, IDN 5390 underwent a 100-fold decrease of activity in taxane-resistant cells (Table 1), while the decrease of activity was less marked for the 7,9-diacylated compounds (**3b** and **3c**), as already observed for taxanes modified by esterification of the hydroxyls of the right-hand upper sector.[16] In light of the constitutional and conformational differences between IDN 5390 and paclitaxel, these observations are surprising and point to a similar mechanism of cytotoxicity. On the other hand, the activity of these acyl derivatives, and especially of the chemically robust carbonates **3c** and **3d**, does not support the view that ring C-secotaxoids are simply metabolic prodrugs of taxanes, undergoing in vivo oxidation of the primary 7-hydroxyl and subsequent spontaneous reallocation to taxanes.[17]

Experimental Section

General Experimental Procedures. Melting points were taken on a Büchi SMP-20 apparatus and are uncorrected. IR spectra were recorded on a Shimadzu DR 8001 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded with a Bruker DRX-500 spectrometer (500 and 125 MHz, respectively) or with a Bruker DRX-300 instrument (300 MHz). The solvent signals ($CHCl_3/CDCl_3$, 7.27/76.9 ppm) were used as internal reference. MS (EI, 70 eV) were taken on VG 7070 EQ spectrometers. Silica gel 60 (70−230 mesh, Merck) was used for open-column chromatography (CC). CH_2Cl_2 and triethylamine were dried by distillation from CaH_2. Organic phases were dried with Na_2SO_4 before evaporation at reduced pressure.

Reaction of C-Secobaccatin 4a with Acetic Anhydride. To a cooled (ice bath) solution of **4a** (274 mg, 0.5 mmol) in dry pyridine (2 mL) was added Ac_2O (191 μL, 206 mg, 2 mmol, 4 molar equiv). After standing in the refrigerator (4 °C) overnight, the reaction was worked up by the addition of a few drops of methanol and then water and EtOAc. The organic phase was washed with brine and evaporated, affording an amorphous residue. This was purified by CC (13 g of silica gel, petroleum ether−EtOAc, 6:4, as eluant) to afford 186 mg (59%) **4c** as a foam: IR ν_{max} (KBr) 3451, 1748, 1731, 1717, 1640, 1625, 1364, 1261, 1250, 1242, 1059 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$−DMSO-d_6, 4:1, 60 °C) δ 8.00 (2H, br s, Bz), 7.66 (1H, t, C-Bz), 7.52 (2H, t, BB′Bz), 5.69 (1H, d, J = 8 Hz, H-2), 5.21 (1H, br m, H-5), 5.04 (1H, d, J = 7.6 Hz, H-20a), 4.73 (1H, br t, J = 7.5 Hz, H-13), 4.52 (1H, d, J = 8 Hz, H-3), 4.32 (1H, br d, J = 8.2 Hz, H-20b), 4.33 (1H, m, H-7a), 4.09 (1H, m, H-7b), 2.50−2.10 (4H, m, H-6a, H-6b, H-14a, H-14b), 2.28 (3H, br s, OAc), 2,22 (3H, br s, OAc), 1.95 (3H, br s, H-19), 1.87 (3H, br s, H-18), 1.20, 1.16 (6H, s, H-16 and H-17); ^{13}C NMR (75 MHz, $CDCl_3$-DMSO-d_6, 4:1, 60 °C) δ 192.6 (s, C-10), 173.0, 170.3, 169.8 (s, OAc), 168.1 (s, Bz), 142.6 (s, C-11), 137.3 (s, C-12), 133. 9 (s, Bz), 129.2 (d, Bz), 129,1 (d, Bz), 128.3 (d, Bz), 86.9 (d, C-5), 86.4 (s, C-4), 79.9 (s, C-1), 75.1 (d, C-2), 73.8 (t, C-20), 70.0 (d, C-13), 62.3 (t, C-7), 44.1 (s, C-15), 36.7, 36.2 (t, C-14 and C-6), 24.8 (q, C-17), 21.9 (q, C-16), 22.8, 22.2, 21.9 (q, OAc), 15.0 (s, C-18), 14.8 (q, C-19) (the signals of C-3, C-8, and C-9 could not be detected); HREIMS m/z $[M]^+$ (628.2534) (calcd for $C_{33}H_{40}O_{12}$, 628.2520).

Reaction of C-Secobaccatin 4a with Di-*tert*-butyl Dicarbonate. To a suspension of **4a** (100 mg, 0.18 mmol) in dry CH_2Cl_2 (1 mL), triethylamine (TEA, 102 μL, 74 mg, 0.37 mmol, 2 molar equiv) were added BOC_2O (191 μL, 206 mg, 0.37 mmol, 2 molar equiv) and DMAP (6 mg, 0.047 mmol, 0.25 molar equiv), resulting into the formation of a colorless solution. After 5 min the reaction was worked up by dilution with EtOAc and by washing with brine. After removal of the solvent, the residue was purified by CC (2.5 silica gel, petroleum ether−EtOAc, 7:3, as eluant) to give 65 mg (47%) of **4d** as a powder: mp 118 °C; ν_{max} (KBr) 3459, 1746, 1669, 1603, 1371, 1279, 1246, 1157, 1126 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.08 (2H, br s, AA′-Bz), ca. 7.60 (3H, BB′-Bz, C-Bz), 5.65 (1H, d, J = 8 Hz, H-2), ca. 5.15 (2H, m, H-5 and H-20a), 4.92 (1H, br t, J = 7.5 Hz, H-13), 4.55 (1H, m H-7b), 4.51 (1H, d, J = 8 Hz, H-3), 4.25 (1H, m, H-20b), 4.15 (1H, m, H-7b), 2.50−2.10 (4H, m, H-6a, H-6b, H-14a, H-14b), 2.28 (3H, br s, OAc), 1.98 (3H, br s, H-19), 1.87 (3H, br s, H-18), 1.83 (3H, s, OAc), 1.59, 1.54 (6H, s, BOC), 1.23, 1.14 (6H, s, H-16 and H-17); HREIMS m/z $[M]^+$ (728.3413) (calcd for $C_{39}H_{52}O_{13}$, 728.3408).

If the reaction was carried out using 1 molar equiv of BOC_2O and TEA and quenched as soon as the starting material had all gone into solution, the 9-mono-BOC derivative **4g** could be obtained as the major reaction product (63%): white powder; mp 139 °C; ν_{max} (KBr) 3494, 1755, 1735, 1667, 1603, 1371, 1275, 1244, 1150, 1127 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.01 (2H, br s, AA′-Bz), ca. 7.60 (1H, t, C-Bz), 7.47 2H, t, BB′-Bz), 5.59 (1H, d, J = 8 Hz, H-2), ca. 5.15 (2H, m, H-5 and H-20a), 4.92 (1H, br t, J = 7.5 Hz, H-13), ca. 4.45 (1H, m, H-20b), 4.39 (1H, d, J = 8 Hz, H-3), ca. 3.70 (2H, m, H-7a, H-7b), 2.50−2.10 (4H, m, H-6a, H-6b, H-14a, H-14b), 1.98 (3H, br s, H-19), 1.87 (3H, br s, H-18), 1.54 (3H, s, BOC), 1.23, 1.14 (6H, s, H-16 and H-17); HREIMS m/z $[M]^+$ (644.2830) (calcd for $C_{34}H_{44}O_{12}$, 644.2833).

Reaction of C-Secobaccatin 4a with Trichloroacetyl Chloride. To a suspension of **4a** (100 mg, 0.18 mmol) in dry CH_2Cl_2 (1 mL) were added triethylamine (TEA, 52 μL, 0.36 mmol, 2 molar equiv) and trichloroacetyl chloride (42 μL, 0.36 mmol, 2 molar equiv). After stirring 1 h at room temperature, the reaction was worked up by dilution with EtOAc and washing with saturated $NaHCO_3$ and brine. After removal of the solvent, the residue was purified by CC (2.5 silica gel, petroleum ether−EtOAc, 95:5, as eluant) to give 89 mg (58%) of **4e** as a powder: ν_{max} (KBr) 3461, 1742, 1669, 1608, 1375, 1269, 1264, 1199, 1164 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.08 (2H, br s, AA′-Bz), ca. 7.66 (1H, t, C-Bz), 7.54 (2H, t, BB′-Bz), 5.65 (1H, d, J = 8 Hz, H-2), ca. 5.15 (2H, m, H-5 and H-20a), 4.97 (1H, br t, J = 7.5 Hz, H-13), ca. 4.50 (1H, m H-7b), 4.38 (1H, d, J = 8 Hz, H-3), ca. 4.30 (2H, m, H-20b and H-7b), 2.50−2.10 (4H, m, H-6a, H-6b, H-14a, and H-14b), 1.99 (3H, br s, H-19), 1.87 (3H, br s, H-18), 1.25, 1.13 (6H, s, H-16 and H-17).

Reaction of C-Secobaccatin 4a with Benzyl Chloroformate. To a suspension of **4a** (300 mg, 0.55 mmol) in dry CH_2Cl_2 (3 mL) were added triethylamine (TEA, 152 mL, 111 mg, 1.1 mmol, 2 molar equiv) and benzyl chloroformate (Cbz-Cl, 156 μL, 187 mg, 1.1 mmol, 2 molar equiv). After stirring at room temperature for 15 min, two further equivalents of

TEA and Cbz-Cl were added. After 5 min, the reaction was worked up by the addition of 2 N H_2SO_4 and dilution with EtOAc. After washing with brine and removal of the solvent, the residue was purified by CC (5 g of silica gel, petroleum ether−EtOAc, 6:4, as eluant) to give 235 mg (63%) of **4h** as a white powder: mp 113−116 °C; ν_{max} (KBr) 3474, 1755, 1728, 1667, 1271, 1232, 1107, 1026, 712 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (2H, br s, AA′-Bz), ca. 7.68 (1H, t, C-Bz), 7.53 (2H, t, BB′-Bz), ca. 7.40 (5H, m, Cbz), 5.59 (1H, d, J = 8 Hz, H-2), 5.28 (2H, br s, Cbz), ca. 5.15 (2H, m, H-5 and H-20a), 4.95 (1H, br t, J = 7.5 Hz, H-13), 4.37 (1H, d, J = 8 Hz, H-3), ca. 4.30 (1H, m, H-20b), ca. 3.65 (2H, m, H-7a, H-7b), 2.50−2.10 (4H, m, H-6a, H-6b, H-14a, and H-14b), 1.98 (3H, br s, H-19), 1.88 (3H, br s, H-18), 1.27, 1.15 (6H, s, H-16 and H-17); HREIMS m/z [M]$^+$ (678.2610) (calcd for $C_{37}H_{42}O_{12}$, 678.2676).

Reaction of 7,9-diTES C-Secobaccatin (4a) with $CeCl_3\cdot 7H_2O$. To a solution of **4b**[3] (500 mg, 0.65 mmol) in MeOH (7.5 mL) was added $CeCl_3\cdot 7H_2O$ (200 mg, 0.54 mmol, 0.83 molar equiv). After stirring 5 min at room temperature, the reaction was worked up by dilution with EtOAc and washing with saturated $NaHCO_3$. The organic phase was washed with brine, dried, and evaporated. The residue was washed with petroleum ether to afford 420 mg (quantitative) of **4f** as a white powder: mp 125 °C; ν_{max} (KBr) 3459, 1746, 1669, 1603, 1371, 1279, 1246, 1157, 1126 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (2H, br s, AA′-Bz), ca. 7.60 (1H, t, C-Bz), 7.46 (2H, t, BB′-Bz), 5.55 (1H, d, J = 8 Hz, H-2), ca. 5.17 (2H, m, H-5 and H-20a), 4.91 (1H, br t, J = 7.5 Hz, H-13), 4.41 (1H, br s, H-3), ca. 4.26 (1H, m, H-20b), ca. 3.70 (2H, br m, H-7a and H-7b), 2.50−2.10 (4H, m, H-6a, H-6b, H-14a, and H-14b), 1.97 (3H, br s, H-19), 1.87 (3H, br s, H-18), 1.20 (6H, br s, H-16 and H-17), ca. 0.95 (9H, m, TES), ca. 0.75 (6H, m, TES); HREIMS m/z [M]$^+$ (658.3187) (calcd for $C_{35}H_{50}O_{10}Si$, 658.3173).

Scheeren−Holton Acetylation of Secobaccatin 4a. To a suspension of **4a** (1.00 g, 1.84 mmol) in THF (11 mL) were added $CeCl_3\cdot 7H_2O$ (90 mg, 0.24 mmol, 0.13 molar equiv) and Ac_2O (2.0 mL, 18.2 mmol, 10 molar equiv). The reaction was stirred at room temperature for 24 h, during which a voluminous precipitate formed. After quenching with the addition of saturated $NaHCO_3$ and EtOAc, the organic phase was washed with brine, dried, and evaporated. The solid residue was purified by CC on silica gel (petroleum ether−EtOAc, 5:5, as eluant) to afford 981 mg of crude **4i**, contaminated by ca. 10% of the 7,10-diacetate **4c** (^{1}H NMR analysis based on the integration of the signals of OH-9 and that of the benzoate ortho-protons). Washing with ether afforded pure **4i** as a white powder: mp 101 °C; ν_{max} (KBr) 3475, 1734, 1655, 1452, 1385, 1275, 1111, 712 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (2H, br s, AA′-Bz), ca. 7.61 (5H, t, C-Bz), 7.48 (2H, t, BB′-Bz), 6.49 (1H, s, 9-OH), 5.59 (1H, d, J = 8 Hz, H-2), ca. 5.17 (2H, m, H-5 and H-20a), 4.90 (1H, br t, J = 7.5 Hz, H-13), 4.39 (1H, br d, J = 8 Hz, H-3), ca. 4.20 (2H, br m, H-20b and H-7a), ca. 4.10 (1H, br m, H-7b), 2.50−2.10 (4H, m, H-6a, H-6b, H-14a, and H-14b), 2.01 (3H, s, OAc), 1.96 (3H, br s, H-19), 1.91 (3H, br s, H-18), 1.26, 1.18 (6H, br s, H-16 and H-17); HREIMS m/z [M]$^+$ (586.2402) (calcd for $C_{31}H_{38}O_{11}$, 586.2414).

Scheeren−Holton Pivaloylation of Secobaccatin 4a. To a suspension of **4a** (300 mg, 0.55 mmol) in THF (3 mL) were added $CeCl_3\cdot 7H_2O$ (ca. 10 mg, catalytic) and pivaloyl chloride (687 μL, 664 mg, 5.5 mmol, 10 molar equiv). The reaction was stirred at room temperature for 5 h and then worked up by the addition of saturated $NaHCO_3$ and EtOAc. The organic phase was washed with brine, dried, and evaporated. The solid residue was purified by CC on silica gel (petroleum ether−EtOAc, 5:5, as eluant) to afford 128 mg of **4l** as a white powder: mp 123 °C; ν_{max} (KBr) 3453, 1726, 1718, 1655, 1277, 1167, 1109, 1071, 712 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (2H, br d, AA′-Bz), ca. 7.65 (1H, t, C-Bz), 7.48 (2H, t, BB′-Bz), 6.50 (1H, s, 9-OH), 5.58 (1H, d, J = 8 Hz, H-2), ca. 5.17 (2H, m, H-5 and H-20a), 4.92 (2H, br t, J = 7.5 Hz, H-13), 4.40−4.05 (4H, overlapping br m, H-3, H-20b, H-7a, and H-7b), 2.50−2.10 (4H, m, H-6a, H-6b, H-14a, and H-14b), 1.99 (3H, br s, H-19), 1.91 (3H, br s, H-18), 1.28, 1.19 (6H, br s, H-16 and H-17), 1.22 (9H, s, OPiv); HREIMS m/z [M]$^+$ (628.2889) (calcd for $C_{34}H_{44}O_{11}$, 628.2884).

Tritylation of 9-Cbz Secobaccatin 4a. To a solution of **4a** (230 mg, 0.34 mmol) in dry pyridine (3.5 mL) were added trityl chloride (472 mg, 1.69 mmol, 5 molar equiv) and DMAP (21 mg, 0.17 mmol, 0.5 molar equiv). After stirring at 40 °C overnight, the reaction mixture was worked up by dilution with 2 N H_2SO_4 and extraction with EtOAc. The organic phase was washed with brine, dried, and evaporated. The residue was purified by CC (5 g of silica gel, petrolem ether−EtOAc, 7:3, as eluant) to afford 235 mg (75%) of **4m** as a white powder: mp 109 °C; ν_{max} (KBr) 3474, 1764, 1719, 1665 1656, 1601, 1449, 1273, 1232, 1071, 1028, 760 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (2H, d, AA′-Bz), ca. 7.60−7.30 (23H, m, BB′- and C-Bz, Cbz, and trityl protons), 5.62 (1H, d, J = 8 Hz, H-2), 5.28 (2H, Cbz), 5.28 (1H, m, H-5), ca. 5.15 (1H, m, H-20a), 4.93 (1H, t, J = 7 Hz, H-13), ca. 4.50 (3H, br s, H-3, H-7a,b and H-20b), 1.94 (6H, br s, H-19 and H-18), 1.27 (3H, s, H-17), 1.21 (3H, s, H-16).

Esterification of the 7,10-Diacyl Derivatives 4c and 4d and of the Monoacyl Derivatives 4i−m with the Protected Amino Acid 5 (Reaction with 4i as Representative). To a solution of **5** (obtained by acidification of 662 mg of its corresponding sodium salt, 1.53 mmol, 6 molar equiv) in dry CH_2Cl_2 (10 mL) were added **4i** (150 mg, 0.25 mmol), EDCI (300 mg, 1.54 mmol, 6 molar equiv), and DMAP (188 mg, 1.54 mmol, 6 molar equiv). After stirring overnight at room temperature, the reaction was worked up by dilution with EtOAc and then addition of saturated $NaHCO_3$. The organic phase was washed with brine, dried, and evaporated. The residue was dissolved in MeOH (10 mL) and treated with 1 mL of methanolic HCl (obtained from the reaction of 560 μL of AcCl in 10 mL of MeOH). After stirring overnight at room temperature, the reaction was worked up by dilution with EtOAc and washing with saturated $NaHCO_3$. The organic phase was washed with brine, dried, and evaporated. The residue was purified by CC (7.5 g of silica gel, petroleum ether−EtOAc,7:3, as eluant) to afford 116 mg of **3e**.

7,9-Diacetyl IDN 5390 (3b): white powder, mp 91 °C; ν_{max} (KBr) 3530, 1740, 1713, 1669, 1368, 1271, 1250, 1115, 712 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, 60 °C) δ 8.03 (2H, d, AA′-Bz), 7.61 (1H, t, C-Bz), 7.48 (2H, t, BB′-Bz), 6.17 (1H, br t, J = 7 Hz, H-13), 5.63 (1H, d, J = 8 Hz, H-2), 5.27 (1H, d, J = 10 Hz, H-5), 5.16 (1H, br d, J = 8 Hz, H-20a), 4.65 (1H, d, J = 9.5 Hz, NH), 4.39 (1H, d, J = 8 Hz, H-3), 4.32 (1H, br d, J = 8 Hz, H-20b), 4.31 (1H, m, H-7a), 4.25 (1H, d, J = 3 Hz, H-2′), 4.18 (1H, m, H-3′), 4.12 (1H, m, H-7b), 2.89 (1H, m, H-14a), 2.46 (2H, m, H-6a and H.-14b), 2.28 (3H, s, OAc), 2.22 (3H, s, OAc), 2.10 (1H, m, H-6b), 1.94 (3H, s, H-19), 1.85 (3H, br s, H-18), 1.60 (1H, m, H-4′a), 1.42 (1H, m, H-4′b), 1.30 (9H, s, BOC), 1.26 (3H, s, H-17), 1.19 (3H, s, H-16), 0.99 (6H, d, J = 7 Hz, H-6′ and H-7′); HREIMS m/z [M]$^+$ (871.3999) (calcd for $C_{45}H_{61}NO_{16}$, 871.3990).

7,9-DiBOC IDN 5390 (3c): white powder, mp 108 °C; ν_{max} (KBr) 3399, 1748, 1713, 1671, 1370, 1279, 1256, 1161, 1127 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, 60 °C) δ 8.07 (2H, d, AA′-Bz), 7.61 (1H, t, C-Bz), 7.52 (2H, t, BB′-Bz), 6.16 (3H, br t, J = 7 Hz, H-13), 5.63 (1H, d, J = 8 Hz, H-2), 5.27 (1H, d, J = 10 Hz, H-5), 5.16 (1H, br d, J = 8 Hz, H-20a), 4.70 (1H, d, J = 9.5 Hz, NH), 4.37 (1H, d, J = 8 Hz, H-3), 4.32 (1H, br d, J = 8 Hz, H-20b), 4.35 (1H, m, H-7a), 4.25 (1H, d, J = 3 Hz, H-2′), 4.18 (1H, m, H-3′), 4.12 (1H, m, H-7b), 2.87 (1H, m, H-14a), 2.48 (2H, m, H-6a and H.-14b), 2.13 (1H, m, H-6b), 1.94 (3H, s, OAc), 1.92 (3H, s, H-19), 1.90 (3H, br s, H-18), 1.63 (1H, m, H-4′a), 1.44 (1H, m, H-4′b), 1.33 (9H, s, BOC), 1.29 (18 H, s, 2 × BOC), 1.28 (3H, s, H-17), 1.19 (3H, s, H-16), 0.99 (6H, d, J = 7 Hz, H-6′ and H-7′); HREIMS m/z [M]$^+$ (987.4813) (calcd for $C_{51}H_{73}NO_{18}$, 987.4828).

9-Cbz IDN 5390 (3d): white powder, mp 108 °C; ν_{max} (KBr) 3453, 1750, 1714, 1670, 1271, 1235, 1179, 1163, 1096 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, 60 °C) δ 8.09 (2H, d, AA′-Bz), 7.63 (1H, t, C-Bz), 7.52 (2H, t, BB′-Bz), ca. 7.44 (5H, m, Cbz), 6.26 (1H, br t, J = 7 Hz, H-13), 5.67 (1H, d, J = 8 Hz, H-2), 5.31 (2H, br s, Cbz), 5.27 (1H, d, J = 10 Hz, H-5), 5.16 (1H, br d, J = 8 Hz, H-20a), 4.80 (1H, d, J = 9.5 Hz, NH), 4.39 (1H, d, J = 8 Hz, H-3), 4.33 (1H, d, J = 8 Hz, H-20b), 4.22 (1H, d, J = 3 Hz, H-2′), 4.18 (1H, m, H-3′), 3.91 (1H, m, H-7a), 3. 76 (1H, m,

H-7b), 2.90 (1H, m, H-14a), 2.48 (2H, m, H-6a and H-14b), 2.16 (1H, m, H-6b), 1.94 (3H, br s, H-19), 1.88 (3H, br s, H-18), 1.33 (9H, s, BOC), 1.28 (3H, s, H-17), 1.20 (3H, s, H-16), 1.02 and 0.99 (6H, d, J = 7 Hz, H-6′ and H-7′); HREIMS m/z $[M]^+$ (921.4159) (calcd for $C_{49}H_{63}NO_{16}$, 921.4147).

9-Acetyl IDN 5390 (3e): white powder, mp 131 °C; foam; ν_{max} (KBr) 3447, 1752, 1713, 1667, 1603, 1395, 1369, 1273, 1100, 1095 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 60 °C) δ 8.04 (2H, d, AA′-Bz), 7.58 (1H, t, C-Bz), 7.47 (2H, t, BB′-Bz), 6.16 (1H, br t, J = 7 Hz, H-13), 5.62 (1H, d, J = 8 Hz, H-2), 5.30 (1H, d, J = 10 Hz, H-5), 5.19 (1H, br d, J = 8 Hz, H-20a), 4.83 (1H, d, J = 9.5 Hz, NH), 4.45 (1H, d, J = 8 Hz, H-3), 4.30 (1H, br d, J = 8 Hz, H-20b), 4.25 (1H, d, J = 3 Hz, H-2′), 4.18 (1H, m, H-3′), 3.91 (1H, m, H-7a), 3.69 (1H, m, H-14a), 2.90 (1H, m, H-14a), 2.46 (2H, m, H-6a and H.-14b), 2.28 (3H, s, OAc), 2.10 (1H, m, H-6b), 1.94 (3H, s, H-19), 1.65 (1H, m, H-4′a), 1.40 (1H, m, H-4′b), 1.33 (9H, s, BOC), 1.27 (3H, s, H-17), 1.21 (3H, s, H-16), 1.02 and 0.99 (6H, d, J = 7 Hz, H-6′ and H-7′); HREIMS m/z $[M]^+$ (829.3876) (calcd for $C_{43}H_{59}NO_{15}$, 829.3885.

Compound 6: white powder, mp 92 °C; ν_{max} (KBr) 3517, 1753, 1713, 1688, 1370, 1285, 1167, 1115, 1094 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.05 (2H, d, AA′-Bz), 7.65 (1H, t, C-Bz), 7.54 (2H, t, BB′-Bz), 5.63 (1H, d, J = 8 Hz, H-2), 5.30 (1H, br s, H-5), 5.01 (1H, br s, H-20a), 4.93 (1H, d, J = 9.5 Hz, NH), 4.38 (1H, d, J = 8 Hz, H-3), 4.30−4.05 (8H, overlapping m, H-20a,b, H-7a,b, H-13, H-5, H-2′, H-3′), 2.80−2.30 (4H, m, H-14a,b and H-6a,b), 1.94 (6H, br s, OAc, H-19 and H-18), 1.33 (9H, s, BOC), 1.27 (3H, s, H-17), 1.21 (3H, s, H-16), 1.18 (9H, s, OPiv), 1.02 and 1.00 (6H, d, J = 7 Hz, H-6′and H-7′); HREIMS m/z $[M]^+$ (871.4361) (calcd for $C_{46}H_{65}NO_{15}$, 871.4359).

Human Tumor Cell Lines. The MCF7-S and MCF7-R (multidrug resistant) human mammary carcinoma cell lines were purchased from the American Type Culture Collection (ATCC), and the MDA435/LCC6-WT and MDR1 cell lines were provided by Dr. R. Clarke, Lombardi Cancer Center, Georgetown University School of Medicine. Cell lines are propagated as monolayers in RPMI-1640 containing 5% FCS, 5% NuSerum IV, 20 mM HEPES, and 2 mM L-glutamine at 37 °C in a 5% CO_2 humidified atmosphere. The doubling times for the cell lines ranged between 20 and 30 h.

Growth Inhibition Assay in 96-Well Microtiter Plates. Assessment of cell growth inhibition was determined according to the methods of Skehan et al.[18] Briefly, cells were plated between 800 and 1500 cells/well in 96-well plates and incubated at 37 °C 15−18 h prior to drug addition to allow cell attachment. Compounds to be tested were solubilized in 100% DMSO and further diluted in RPMI-1640 containing 10 mM HEPES. Each cell line was treated with 10 concentrations of compound (5 log range). After a 72 h incubation, 100 μL of ice-cold 50% TCA was added to each well and incubated for 1 h at 4 °C. Plates were then washed five times with tap water to remove TCA, low molecular weight metabolites, and serum proteins. Then, 50 μL of 0.4% sulforhodamine B (SRB), an anionic protein stain, was added to each well. At cell densities ranging from very sparse to supraconfluent, SRB staining changed linearly with increases or decreases in number of cells and protein concentrations. These staining characteristics provided an accurate assessment of cell growth.[18] Following a 5 min incubation at room temperature, plates were rinsed five times with 0.1% acetic acid and air-dried. Bound dye was solubilized with 10 mM Tris base (pH 10.5) for 5 min on a gyratory shaker. Optical density was measured at 570 nm.

Data Analysis. Data were fit with the Sigmoid-Emax concentration-effect model[19] with nonlinear regression and weighted by the reciprocal of the square of the predicted response. The fitting software was developed at RPCI with MicroSoft FORTRAN and used the Marquardt algorithm[20] as adapted by Nash[21] for the nonlinear regression. The concentration of drug that resulted in 50% growth inhibition (IC_{50}) was calculated.

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