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Synthesis and biological evaluation of methoxylated analogs of the newer generation taxoids IDN5109 and IDN5390

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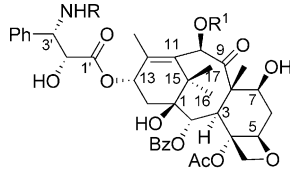
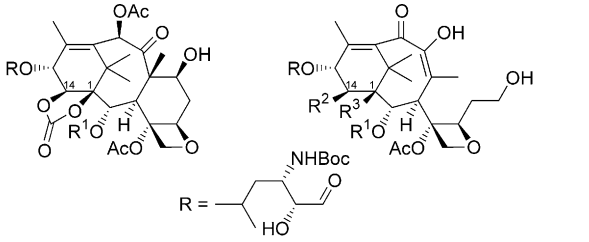
Abstract—Starting from 10-deacetylbaccatin III (**7**), the 2-debenzoyl-2-*m*-methoxybenzoyl analogs of the newer generation taxoids IDN5109 (**3**) and IDN5390 (**4**) were synthesized. The biological evaluation of these compounds (**5** and **6**, respectively) showed a general increase of cytotoxicity, as observed in first-generation anticancer taxanes.

Paclitaxel (**1**), a natural product first isolated from the bark of *Taxus brevifolia*, and its semi-synthetic derivative docetaxel (**2**), are currently used in the treatment of a large number of human tumors such as ovarian cancer, breast cancer, melanoma, and nonsmall cell lung cancer. Their mechanism of action involves stabilization of microtubules and inhibition of tubulin depolymerization.[1] Paclitaxel and docetaxel suffer from a series of disadvantages, including a poor water solubility and the quick development of resistance,[2] that have fuelled the search of analogs endowed with a better clinical profile.[3]



1: R = Bz, R^1 = Ac (paclitaxel)
2: R = boc, R^1 = H (docetaxel)

The norstatin esters IDN5109 (**3**)[3] and IDN5390 (**4**)[4] have recently emerged as interesting clinical candidates to overcome resistance to paclitaxel and to allow oral administration. When assayed against paclitaxel-responsive tumors, the activity of **3** and **4** was basically in the range of the first-generation taxoids.[5] Since the introduction of an azido- or a methoxy *meta*-substituent on the 2-benzoate has been reported to magnify the cytotoxicity of taxoids,[6] we have investigated this effect in the newer generation taxoids **3** and **4**, hoping to improve their cytotoxic potency while substantially retaining the assets of the lead structures in terms of solubility and spectrum of activity.



3: R^1 = Bz (IDN5109)
4: R^1 = Bz, R^2 = H, R^3 = OH (IDN5390)
5: R^1 = *m*-MeOBz
6: R^1 = *m*-MeOBz, R^2,R^3 = 1,14-carbonate

Synthesis of the IDN5109 analog **5** started from the enone **8**, in turn available from 10-deacetylbaccatin III (**7**) in five steps (Scheme 1).[7]

Keywords: Paclitaxel; IDN5109; IDN5390; Methoxylated; Newer generation taxoids.
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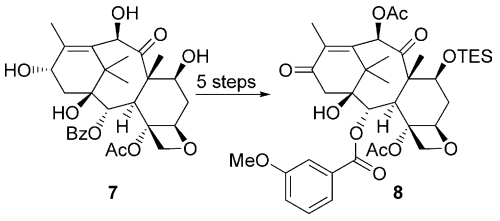
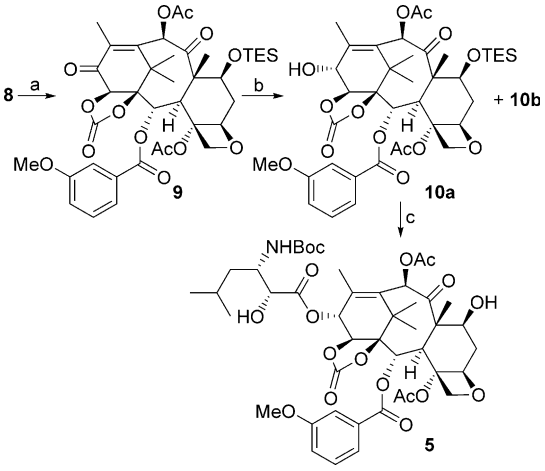


Scheme 1.

For the preparation of compound **10** we capitalized on the recently reported diastereoselective 14β-hydroxylation of 13-oxotaxoids.[8] Thus, treatment of **8** with 2-benzenesulfonyl-3-(3-nitrophenyl)oxaziridine, in the presence of ^{t}BuOK, followed by in situ carbonylation with carbonyldiimidazole gave compound **9** (Scheme 2).[9]



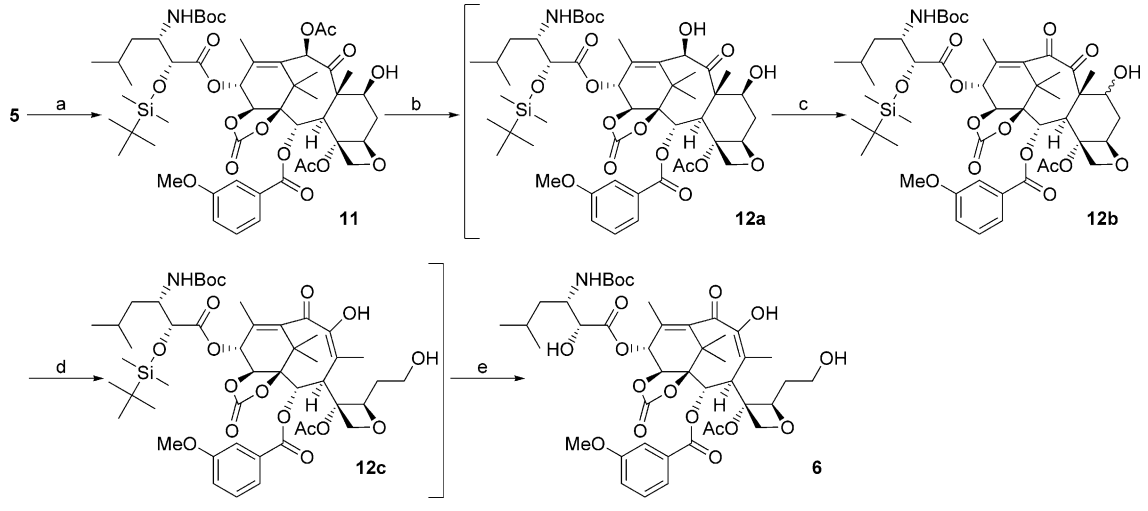
Scheme 2. Reagents and conditions: (a) i—^{t}BuOK, 2-benzenesulfonyl-3-(3-nitrophenyl)oxaziridine, THF, DMPU, −65 °C to −55 °C, 1.5 h; ii—carbonyldiimidazole, imidazole, DCM, 40 °C, 1.5 h, 30%; (b) $Et_4N(BH_4)$, MeOH, −40 °C to 0 °C, 2.5 h, 60%; (c) i—*N*-Boc (4*S*,5*R*)-2-(2,4-dimethoxyphenyl)-4-phenyl-5-oxazolidine carboxylic acid, DMAP, EDC, DCM, rt, 1.5 h; ii—HF, pyridine, CH_3CN, 0 °C to rt, 2 h; iii—HCl 0.6 N (MeOH), DCM, 0 °C, 5 h, 55%.

Reduction of the 13-keto group of **9** with $Et_4N(BH_4)$[8] (Scheme 2) gave a mixture the α- and β-epimers (**10a** and **10b**, respectively), that could be isolated by column chromatography.[10] The configuration of C13 and C14 of compounds **10a** and **10b** was assigned on the basis of the NOESY1D spectra. Compound **10a** showed an enhancement of the H-17 signal upon selective saturation of H-13, whereas irradiation of H-14 showed a NOE correlation with H-3. Selective saturation of H-14 in **10b** showed an enhancement of the H-13 and H-3 signals, which indicates that both H-14 and H-13 lie on the α-face of the molecule. With **10a** in hand, the completion of the synthesis was easily accomplished by coupling with *N*-Boc (4*S*,5*R*)-2-(2,4-dimethoxyphenyl)-4-phenyl-5-oxazolidine carboxylic acid[4] and global deprotection of the crude esterification product (Scheme 2).[11]

IDN5390 (**4**) is a ring *C*-seco taxoid, and the synthesis of its methoxylated analog was carried out according to a general retro-aldol entry for this type of compound.[12] Thus, compound **5** was first protected at C2′ with TBDMSCl[13] to afford **11**,[14] next converted to the *C*-seco derivative **6** in four steps (Scheme 3)[15] that could be carried out without purification of any intermediate. Thus, **11** was deacetylated at C10 with hydrazine, and the ketol function obtained was oxidized with $Cu(OAc)_2$.[12] Reductive trapping of the ring *C*-seco tautomer was obtained by treatment with L-Selectride.[12] Final deprotection with TBAF gave **6** in a 48% overall yield.

The cytotoxicity of **5** and **6** was investigated on MCF7 and MCF7/R cell lines (Table 1).[16] As expected, the *meta*-methoxy group had a general boosting effect on cytotoxicity, both on MCF7 and MCF7/R cell lines, showing that the '*meta*-effect' (enhanced interaction between the C-2 ring and asp224 in the β-tubulin binding pocket)[17] has broad generality in anticancer taxoids.

In conclusion, we have demonstrated that the insertion of a *meta* methoxy group on the C-2 benzoate leads to



Scheme 3. Reagents and conditions: (a) TBDMSCl, DMAP, DCM, rt, overnight, 90%; (b) $N_2H_4{\cdot}H_2O$, EtOH 96%, −15 °C, 45 min; (c) $Cu(OAc)_2{\cdot}H_2O$, MeOH, rt, overnight; (d) L-Selectride, THF, −5 °C, instantaneous; (e) TBAF, THF, 0 °C, 20 min; overall yield (b + c + d + e) 48%.

Table 1. Cytotoxic potency of **5** and **6** in comparison with **1**, **3**, and **4** IC50[a]

Compound	72 h cell drug exposure ± SE (SRB test)[b]		
	MCF7[c]	MCF7/R[c]	RI[d]
1	0.6 ± 0.08	585 ± 62	975
3	1.6 ± 0.1	32 ± 2.4	20
4	11.2 ± 0.81	1044 ± 93	93
5	0.24 ± 0.04	4 ± 0.8	17
6	0.5 ± 0.05	120 ± 30	240

[a] IC_{50}: concentration inhibiting the 50% of cellular growth compared to untreated cells in nanomoles.

[b] Cells were exposed for 72 h to the drugs and the IC_{50} was determined by SRB metabolic test. Values of IC_{50} (means of at least three independent experiments) are reported ± standard error.

[c] Cell lines: MCF7 human mammary carcinoma; MCF7/R: subline with acquired resistance to doxorubicin (DX).

[d] RI, resistance index; IC_{50} resistant line/IC_{50} sensitive line.

a general increase of cytotoxicity also in newer generation taxoids, including those of the ring *C*-seco type that differ substantially from taxanes in terms of topology and conformational properties. While having minimal effect on water solubility, the introduction of a methoxyl can in principle have dramatic effects on drug metabolism, and pharmacokinetics data are clearly needed to assess the overall effect of this maneuver on the clinical profile of newer generation taxoids.

Acknowledgments

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References and notes

1. Horwitz, S. B.; Cohen, D.; Rao, S.; Ringel, I.; Shen, H.-J. *Monogr. Natl. Cancer Inst.* **1993**, *15*, 55.
2. Rowinsky, E. K.; Donehower, R. C. *N. Engl. J. Med.* **1995**, *332*, 1004.
3. (a) Eckstein, J. W. *IDrugs* **2004**, *7*, 575; (b) Miller, M. L.; Ojima, I. *Chem. Rec.* **2001**, *1*, 195.
4. Appendino, G.; Danieli, B.; Jakupovic, J.; Belloro, E.; Scambia, E.; Bombardelli, E. *Tetrahedron Lett.* **1997**, *38*, 4273.
5. (a) Nicoletti, M. I.; Colombo, T.; Rossi, C.; Monardo, C.; Stura, S.; Zucchetti, M.; Riva, A.; Morazzoni, P.; Donati, M. B.; Bombardelli, E.; D'Incalci, M.; Gavazzi, R. *Cancer Res.* **2000**, *60*, 842; (b) Ferlini, C.; Raspaglio, G.; Mozzetti, S.; Cicchillitti, L.; Filippetti, F.; Gallo, D.; Fattorusso, C.; Campiani, G.; Scambia, G. *Cancer Res.* **2005**, *65*, 2397.
6. Chaudhary, A. G.; Gharpure, M.; Rimoldi, J. M.; Chordia, M. D.; Gunatilaka, A. A. L.; Kingston, D. G. I. *J. Am. Chem. Soc.* **1994**, *116*, 4097.
7. (a) Chordia, M. D.; Kingston, D. G. I. *Tetrahedron* **1997**, *53*, 5699; (b) Appendino, G. J.; Jakupovic, J.; Cravotto, G.; Enriù, R.; Varese, M.; Bombardelli, E. *Tetrahedron Lett.* **1995**, *36*, 3233.
8. Baldelli, E.; Battaglia, A.; Bombardelli, E.; Carenzi, G.; Fontana, G.; Gambini, A.; Gelmi, M. L.; Guerrini, A.; Pocar, D. *J. Org. Chem.* **2003**, *68*, 9773.
9. Synthetic procedure for **9**: a suspension of *t*-BuOK (450 mg, 4 mmol) in THF (2.5 mL) was stirred at rt for 10 min, cooled at −65 °C, and then a solution of **8** (729 mg, 1.0 mmol) in THF/DMPU (8:3, 11 mL) was added in 2 min. After 15 min, a solution of 2-benzenesulfonyl-3-(3-nitrophenyl)oxaziridine (0.86 g, 2.8 mmol) in THF/DMPU (9:1, 10 mL) was added in 3 min. and the temperature was slowly raised (about 1 h) to −55 °C. At this point, acetic acid (0.6 mL) and aqueous NH_4Cl (10%, 25.0 mL) were sequentially added, and the temperature of the mixture was allowed to rise to rt under stirring. The organic layer was extracted with water, dried, and evaporated. The crude product was dissolved in anhydrous DCM (20 mL), and carbonyldiimidazole (2.3 g, 22 mmol) and imidazole (734 mg, 3.2 mmol) were sequentially added. The solution was stirred at 40 °C for 1.5 h and then diluted with DCM (200 mL), washed with HCl 5%, $NaHCO_3$ 5%, water, and brine, dried, and the solvent was evaporated. Chromatography of the residue (SiO_2, hexane/EtOAc, 8:2) gave compound **9** (230 mg, 0.3 mmol, 30%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.56 (q, J = 7.8 Hz, 6H), 0.90 (t, J = 7.8 Hz, 9H), 1.16 (s, 3H), 1.34 (s, 3H), 1.70 (s, 3H), 1.89 (m, 1H), 2.17 (s, 3H), 2.21 (s, 6H), 2.52 (m, 1H), 3.77 (d, J = 6.9 Hz, 1H), 3.83 (s, 3H), 4.18 (d, J = 8.7 Hz, 1H), 4.35 (d, J = 8.7 Hz, 1H), 4.44 (m, 1H), 4.75 (s, 1H), 4.89 (br d, J = 7.8 Hz, 1H), 6.08 (d, J = 6.9 Hz, 1H), 6.49 (s, 1H), 7.14 (br d, J = 8.7 Hz, 1H), 7.37 (t, J = 8.0 Hz, 1H), 7.49 (br s, 1H), 7.54 (d, J = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 5.2, 6.7, 9.8, 13.9, 19.3, 20.7, 21.7, 32.7, 36.9, 41.6, 45.4, 55.4, 59.2, 68.3, 72.0, 74.6, 75.8, 77.1, 80.5, 83.8, 86.4, 114.7, 120.7, 122.0, 128.9, 130.0, 139.3, 151.1, 151.3, 159.8, 164.3, 168.7, 170.2, 190.8, 199.0; MS (ESI) *m/z* 793 ($M+Na^+$).
10. Synthetic procedure for **10a** and **10b**: To a solution of **9** (771 mg, 1 mmol) in MeOH (15 mL) at −40 °C, $Et_4N(BH_4)$ (1.74 g, 12 mmol) in MeOH (5 mL) was added, and the reaction mixture was stirred at −40 °C for 30 min. The temperature was then raised to 0 °C and, after stirring for 2 h, citric acid (1.5 g) and a saturated solution of citric acid in water (7 mL) were sequentially added. The temperature was allowed to raise to rt and the reaction mixture was extracted with DCM, dried, and evaporated. The crude material was purified by gravity chromatography (SiO_2, hexane/EtOAc, 3:2) to yield **10a** (464 mg, 0.60 mmol, 76%) and **10b** (147 mg, 0.19 mmol, 19%). **10a**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.55 (q, J = 7.8 Hz, 6H), 0.89 (t, J = 7.8 Hz, 9H), 1.12 (s, 3H), 1.29 (s, 3H), 1.67 (s, 3H), 1.86 (m, 1H), 2.17 (s, 3H), 2.18 (s, 3H), 2.28 (s, 3H), 2.51 (m, 1H), 3.69 (d, J = 7.5 Hz, 1H), 3.83 (s, 3H), 4.16 (d, J = 8.0 Hz, 1H), 4.31 (d, J = 8.0 Hz, 1H), 4.44 (m, 1H), 4.77 (d, J = 5.7 Hz, 1H), 4.93 (br d, J = 7.5 Hz, 1H), 4.98 (br d, J = 5.7 Hz, 1H), 6.04 (d, J = 7.2 Hz, 1H), 6.41 (s, 1H), 7.13 (br d, J = 7.2 Hz, 1H), 7.35 (t, J = 8.1 Hz, 1H), 7.53 (br s, 1H), 7.59 (d, J = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 5.2, 6.7, 10.0, 14.8, 20.8, 21.6, 22.3, 25.9, 37.0, 41.4, 46.6, 55.4, 58.7, 69.4, 71.9, 72.0, 75.0, 76.0, 80.3, 83.9, 84.1, 88.4, 114.5, 120.5, 122.1, 129.4, 129.9, 132.8, 142.2, 152.8, 159.7, 164.7, 169.2, 170.4, 200.8; MS (ESI) *m/z* 795 ($M+Na^+$). **10b**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.58 (q, J = 7.8 Hz, 6H), 0.892 (t, J = 7.8 Hz, 9H), 1.29 (s, 3H), 1.42 (s, 3H), 1.71 (s, 3H), 1.90 (m, 1H), 2.20 (s, 3H), 2.25 (s, 3H), 2.31 (s, 3H), 2.51 (m, 1H), 3.67 (d, J = 7.3 Hz, 1H), 3.85 (s, 3H), 4.20 (d, J = 8.8 Hz, 1H), 4.34 (d, J = 8.8 Hz, 1H), 4.42 (m, 2H), 4.80 (d, J = 7.8 Hz, 1H), 4.90 (br d, J = 8.8 Hz, 1H), 6.10 (d, J = 7.3 Hz, 1H), 6.43 (s, 1H), 7.15 (br d, J = 7.8 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 7.53 (br s, 1H), 7.58 (d, J = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 5.2, 6.7, 9.9, 19.9, 20.8, 21.3, 22.0, 30.4, 37.0, 40.7, 46.1, 55.4, 59.1,

67.7, 68.7, 71.8, 74.8, 75.0, 76.0, 80.9, 83.9, 92.3, 114.8, 120.3, 122.0, 129.3, 129.9, 138.7, 139.9, 152.7, 159.8, 164.5, 169.0, 170.1, 200.5; MS (ESI) *m/z* 795 ($M+Na^+$).

11. Synthetic procedure for **5**: to a solution of *N*-Boc (4*S*,5*R*)-2-(2,4-dimethoxyphenyl)-4-phenyl-5-oxazolidine carboxylic acid (obtained by acidification of 0.75 g of its corresponding sodium salt, 1.75 mmol) in dry DCM (5 mL), a solution of **10a** (386 mg, 0.5 mmol) in dry DCM, EDCI (288 mg, 1.5 mmol), and DMAP (183 mg, 1.5 mmol) was added. After stirring at rt for 1.5 h, the reaction was worked up by dilution with EtOAc and then addition of sat. $NaHCO_3$. The organic phase was washed with brine, dried, and evaporated. The residue was dissolved in CH_3CN/pyridine, (5:4, 5 mL), cooled to 0 °C, and HF (70% pyridine solution, 3.5 mL) was added. After stirring at rt for 2 h, the solution was diluted with EtOAc and washed with $KHSO_4$ (10%), $NaHCO_3$ 5%, water, and brine. The organic phase was evaporated, the residue was dissolved in dry DCM (8 mL), cooled to 0 °C, and treated with 0.25 mL of methanolic HCl (obtained from the reaction of 9 μL AcCl in 0.25 mL MeOH). After stirring overnight at room temperature, the reaction was worked up by dilution with DCM and washing with sat. $NaHCO_3$. The organic phase was washed with water and brine, dried, and evaporated. The residue was purified by gravity column chromatography (SiO_2, hexane/EtOAc, 1:1) to afford **5** (248 mg, 55%). Amorphous solid; $[\alpha]_D^{20} - 39.6°$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 0.94 (d, $J = 6.7$ Hz, 3H), 0.97 (d, $J = 6.7$ Hz, 3H), 1.26 (s, 3H), 1.30–1.34 (m, 1H), 1.33 (s, 3H), 1.34 (s, 9H), 1.69 (m, 2H), 1.70 (s, 3H), 1.87 (m, 1H), 1.88 (s, 3H), 2.23 (s, 3H), 2.46 (s, 3H), 2.54 (m, 1H), 3.68 (d, $J = 7.3$ Hz, 1H), 3.84 (s, 3H), 4.08 (m, 1H), 4.20 (d, $J = 8.3$ Hz, 1H), 4.30 (m, 1H), 4.32 (d, $J = 8.3$ Hz, 1H), 4.37 (m, 1H), 4.73 (d, $J = 9.8$ Hz, 1H), 4.83 (d, $J = 6.7$ Hz, 1H), 4.93 (br d, $J = 9.8$ Hz, 1H), 6.08 (d, $J = 7.3$ Hz, 1H), 6.25 (s, 1H), 6.44 (br d, $J = 6.7$ Hz, 1H), 7.13 (dd, $J = 7.9$, 3.0 Hz, 1H), 7.36 (t, $J = 7.9$ Hz, 1H), 7.54 (br s, 1H), 7.61 (d, $J = 7.9$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.7, 15.0, 20.8, 22.1, 22.5, 23.1, 23.2, 24.8, 26.0, 28.2, 35.4, 40.5, 41.8, 45.0, 51.6, 55.4, 58.7, 69.5, 71.7, 73.9, 74.6, 74.8, 76.0, 79.6, 80.4, 80.5, 84.2, 88.1, 114.0, 121.0, 122.3, 129.1, 130.0, 133.5, 139.8, 151.8, 156.2, 159.8, 164.7, 170.6, 170.9, 173.0, 202.2; MS (ESI) *m/z* 924 ($M+Na^+$); HRMS *m/z* calcd for $C_{45}H_{59}NO_{18}Na^+$ 924.3630, found 924.3636.
12. Appendino, G.; Noncovich, A.; Bettoni, P.; Dambruoso, P.; Sterner, O.; Fontana, G.; Bombardelli, E. *Eur. J. Org. Chem.* **2003**, 4422.
13. Gunatilaka, A. A. L.; Ramdayal, F. D.; Sarragiotto, M. H.; Kingston, D. G. I.; Sackett, D. L.; Hamel, E. *J. Org. Chem.* **1999**, *64*, 2694.
14. **11**: ^1H NMR ($CDCl_3$, 300 MHz): δ 0.11 (s, 3H), 0.15 (s, 3H), 0.86 (s, 3H), 0.91 (s, 3H), 0.95 (s, 9H), 1.27 (s, 3H), 1.30-1.34 (m, 1H), 1.36 (s, 9H), 1.45 (s, 3H), 1.66 (m, 2H), 1.71 (s, 3H), 1.90 (m, 1H), 1.91 (s, 3H), 2.25 (s, 3H), 2.50 (s, 3H), 2.56 (m, 1H), 3.71 (d, $J = 7.5$ Hz, 1H), 3.88 (s, 3H), 4.16 (m, 1H), 4.23 (d, $J = 8.4$ Hz, 1H), 4.33 (m, 2H), 4.40 (m, 1H), 4.52 (d, $J = 9.9$ Hz, 1H), 4.85 (d, $J = 6.9$ Hz, 1H), 4.95 (br d, $J = 7.2$ Hz, 1H), 6.10 (d, $J = 7.5$ Hz, 1H), 6.27 (s, 1H), 6.38 (br d, $J = 6.9$ Hz, 1H), 7.15 (dd, $J = 8.4$, 2.7 Hz, 1H), 7.37 (t, $J = 8.4$ Hz, 1H), 7.55 (br s, 1H), 7.61 (d, $J = 8.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ −5.1, −4.6, 9.9, 15.4, 21.0, 22.2, 23.0, 23.6, 23.7, 24.9, 25.9, 26.1, 28.4, 35.7, 41.7, 42.0, 45.3, 51.8, 55.6, 58.8, 69.7, 71.8, 74.5, 75.0, 75.3, 76.1, 79.6, 79.9, 80.7, 84.5, 88.3, 114.3, 121.2, 122.4, 129.3, 130.2, 133.6, 140.1, 152.0, 155.4, 160.0, 164.9, 170.8, 171.1, 171.9, 202.4; MS (ESI) *m/z* 1038 ($M+Na^+$).
15. Synthetic procedure for **6**: To a solution of **11** (100 mg, 0.1 mmol) in EtOH (10 mL), hydrazine monohydrate (25 mg, 0.5 mmol) was added at −15 °C. After stirring for 45 min, the reaction mixture was worked up by dilution with EtOAC, washing with saturated NH_4Cl and brine, and concentration under reduced pressure to give crude **12a**, which was used for the next step. Thus, **12a** was dissolved in MeOH (3 mL), $Cu(OAc)_2 \cdot H_2O$ (974 mg) was added, and the suspension was magnetically stirred for 24 h. The reaction mixture was worked up by concentration under reduced pressure, filtration through Celite, and washing of the cake with EtOAc. The organic phase was washed with 2% NH_3 and brine, and dried, giving the epimeric mixture **12b** that was directly employed for the reductive fragmentation step. **12b** was then dissolved in dry THF (ca. 2 mL), cooled to −15 °C, and a solution of L-selectride (1.0 M, 0.2 mL, and 0.2 mmol) was added dropwise. At the end of the addition, the yellow solution was diluted with EtOAc and washed with 2 N H_2SO_4 and brine. After evaporation of the solvent, the residue was dissolved in dry THF (2 mL), cooled to 0 °C, and treated with tetrabutylammonium fluoride (TBAF, 1 M in THF, 0.2 mL, and 0.2 mmol). After stirring for 20 min, the reaction mixture was diluted with EtOAC, washed with 2N H_2SO_4 and brine, dried, and evaporated. The residue was purified by chromatography (SiO_2, hexane/EtOAc, 1:1) to give **6** (40 mg, 48%). Amorphous solid; $[\alpha]_D^{20} - 20.5°$ (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz, 50 °C): δ 0.98 (d,$J = 6.0$ Hz, 6H), 1.27 (s, 3H), 1.34 (s, 9H), 1.37 (s, 3H), 1.49 (m, 1H), 1.58 (m, 1H), 1.70 (m, 1H), 1.91 (s, 3H), 1.94 (m, 1H), 1.99 (br s, 6H), 2.37 (m, 1H), 3.71 (m, 1H), 3.85 (s, 3H), 3.87 (m, 1H), 4.20 (m, 2H), 4.29 (d, $J = 8.1$ Hz, 1H), 4.33 (d, $J = 3.6$ Hz, 1H), 4.78 (d, $J = 9.9$ Hz, 1H), 5.14 (m, 1H), 5.24 (br d, $J = 10.8$ Hz, 1H), 5.42 (br s, 1H), 6.05 (d, $J = 9.3$ Hz, 1H), 6.49 (br d, $J = 3.3$ Hz, 1H), 7.15 (dd, $J = 8.1$, 2.4 Hz, 1H), 7.39 (t, $J = 8.1$ Hz, 1H), 7.48 (br s, 1H), 7.57 (d, $J = 8.1$ Hz, 1H); MS (ESI) *m/z* 882 ($M + Na^+$). HRMS *m/z* calcd for $C_{43}H_{57}NO_{17}Na^+$ 882.3524, found 882.3531.
16. **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. (Skehan, P.; Streng, R.; Scudierok, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, 82, 1107). 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 the 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 (Holford, N.H. G; Scheiner, L. B. *Clin. Pharmocokin.* **1981**, 6, 429) 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 uses the Marquardt algorithm (Marquardt, D. W. *J. Soc. Ind. Appl. Math.* **1976**, 11, 431) as adapted by Nash (Nash, J. C. *Compact numerical method for computers: Linear algebra and function minimization*; John Wiley & Sons: New York, 1979) for the nonlinear regression. The concentration of drug which resulted in 50% growth inhibition (IC_{50}) was calculated.

17. (a) Ganesh, T.; Guza, R. C.; Bane, S.; Ravinfra, R.; Shanker, N.; Lakdawala, A. S.; Snyder, J. P.; Kingston, D. G. I. *PNAS* **2004**, *101*, 10006; (b) Snyder, J. P.; Nettles, J. H.; Cornett, B.; Downing, K. H.; Nogales, E. *PNAS* **2001**, *98*, 5312; (c) He, L.; Jagtap, P. G.; Kingston, D. G. I.; Shen, H.-J.; Orr, G. A.; Horwitz, S. B. *Biochemistry* **2000**, *39*, 3972.