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Design, Synthesis and Biological Activity of Novel C2-C3′ N-Linked Macrocyclic Taxoids

Iwao Ojima,[a,*] Xudong Geng,[a] Songnian Lin,[a] Paula Pera[b] and Ralph J. Bernacki[b]

[a]*Department of Chemistry, State University of New York at Stony Brook, NY 11794-3400, USA*
[b]*Department of Experimental Therapeutics, Rosewell Park Cancer Institute, Elm and Carton Streets, Buffalo, NY 14263, USA*

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Abstract—A series of novel macrocyclic taxoids was designed and synthesized by connecting the C-2 and C-3′ N positions of the taxoid framework with various tethers. Cytotoxicity of these macrocyclic taxoids was evaluated against a human breast cancer cell line LCC6-WT, and a couple of the taxoids exhibited 0.09–0.3 μM IC_{50} values.

Taxol® (paclitaxel)[1] and its semi-synthetic analogue Taxotère® (docetaxel)[2] are two extremely important chemotherapeutic drugs widely used today. These drugs act by facilitating tubulin polymerization and stabilizing the resulting microtubules, thereby inhibiting the normal dynamic reorganization of microtubular network required for mitosis, which eventually induces apoptosis.[3] This mechanism of action remained unique until several structurally dissimilar natural products (epothilones,[4,5] eleutherobin,[6] discodermolide,[7] and laulimalide[8,9]) were found recently to share the same mechanism of action. In order to account for this intriguing finding, a possible common pharmacophore for those microtubule-stabilizing agents was proposed by these laboratories.[10] Based on this common pharmacophore model, a series of novel macrocyclic taxoids bearing a linker connecting the C-2 and C-3′ positions was synthesized[10,11] as potential hybrids of nonataxel and epothilone B. These 'C-linked' macrocyclic taxoids were found to be moderately cytotoxic against a human breast cancer cell line LCC6.[10,11]

In the first common pharmacophore proposal, a 'hydrophobic clustering' conformation of nonataxel was employed, which was the prevalent conformation in aqueous media.[10] However, the electron crystallographic structure of paclitaxel-bound Zn^{2+}-stabilized α,β-tubulin dimer (3.7 Å resolution) by Nogales et al.[12] incorporated a docetaxel molecule with a conformation close to that found in its X-ray crystallographic analysis.[13] Accordingly, we became interested in designing and synthesizing a new series of macrocyclic taxoids, mimicking the tubulin-bound docetaxel structure proposed by Nogales et al.[12] Thus, we designed novel N-linked macrocyclic taxoids **1** connecting the C-2 carboxylate with the C-3′ N acyl moiety with various linkers (Fig. 1). We report here the synthesis and biological activity of these novel 'N-linked' macrocyclic taxoids **1**.

In designing the synthetic routes to the designed N-linked macrocyclic taxoids, the ring-closing metathesis (RCM)[14] was chosen for the key step based on our successful use of this method in the syntheses of the C-linked series taxoids.[10,11] The retrosynthetic analysis for the construction of N-linked macrocyclic taxoids is shown in Scheme 1. As Scheme 1 illustrates, N-linked macrocyclic taxoids **1** can be obtained from diene precursor **2** by RCM. Using our β-lactam ring-opening coupling protocol,[15–20] the diene precursor **2** can be synthesized by coupling C-2 modified baccatin **4** with β-lactam **3** bearing a terminal alkenyl tether. Baccatin **4** can be easily prepared from 2-debenzoyl-7,10,13-tri-TES-baccatin **5** that should be readily derived from the

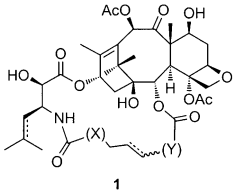

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Figure 1. 'N-Linked' macrocyclic taxoids **1**.

*Corresponding author. Tel.: +1-631-632-7947; fax: +1-631-632-7942; e-mail: iojima@notes.cc.sunysb.edu

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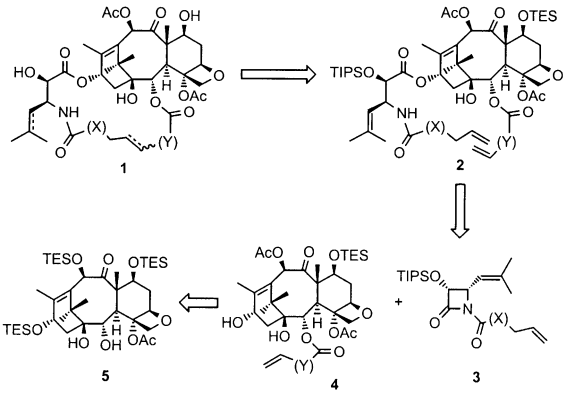
naturally occurring 10-deacetylbaccatin III (DAB). The enantiopure β-lactam **3** can be prepared through highly efficient chiral enolate-imine cyclocondensation,[15,19–21] followed by *N*-acylation.

The synthesis started with 2-debenzoyl-10-deacetyl-7,10,13-tri-TES-baccatin **5**, which was prepared from 10-DAB by the previously reported method.[11,22] Esterification of **5** with 3-vinylbenzoic acid (**6a**) or (3*R*)-3-methylhept-6-enoic acid (**6b**) in the presence of DIC and DMAP gave desired C-2-modified baccatins **7a** and **7b**, respectively. It should be noted that the formation of a considerable amount of D-ring-opened byproduct[11,23] (36–50%) was observed when using acid **6a**. Optimization for this particular reaction is in progress. Removal of all silyl protecting groups of **7a**,**b** by HF-pyridine, followed by selective protection of C-7 hydroxyl group with TES gave 2-modified-7-TES-10-deacetylbaccatin (**8a**,**b**) in excellent yield. Subsequent selective C10 acetylation at −40 °C using LiHMDS as base gave C-2-modified baccatin **4a**,**b** in good yield (Scheme 2).

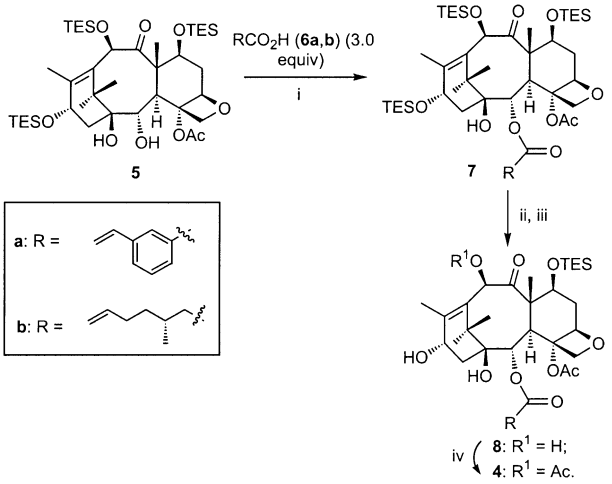
Enantiopure β-lactams **3a–c** were prepared in high yields by acylation of *N*-H-β-lactam **9**[24] with allyl chlorofomate (**10a**), pent-4-enoyl chloride (**10b**), and but-3-enyl chloroformate (**10c**) (Scheme 3).

Ring-opening coupling of β-lactams **3a–c** with C-2-modified baccatin **4a** using LiHMDS in THF at −40 °C gave dienes **2a–c** in 70–80% yield. The diene precursors **2a–c** were subjected to RCM using the Grubbs catalyst[14] in CH_2Cl_2 to afford the macrocyclic taxoids **11a–c** in 70–96% yield. It is worthy of note that the formation of only *E*-isomers of **11a–c** was observed in these RCM reactions. Removal of silyl protecting groups gave macrocyclic taxoids **1a–c** in 74–80% yields (Scheme 4). Hydrogenation of macrocyclic taxoids **1a–c**, thus obtained, on Pd/C afforded the corresponding macrocyclic taxoids **1(H)a–c** bearing a saturated linker and 2-methylpropyl group at C-3′ (Scheme 4).

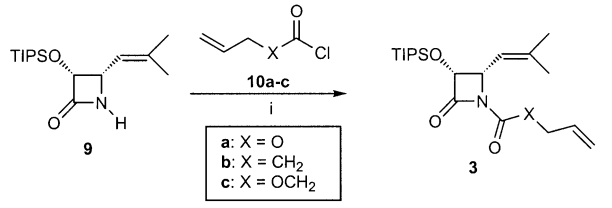
In a similar manner, C-2-modified baccatin **4b** was coupled with β-lactam **3a**,**b** to give dienes **2d** and **2e** in 93 and 77% yields, respectively. The RCM reactions of **2d** and **2e** afforded **11d** ($E/Z = 3.5$, 91%) and **11e** ($E/Z = 2$, 93%) in high yields. The silyl protecting groups of **11d**,**e** were removed by HF-pyridine and the *E* and *Z* isomers of the resulting macrocyclic taxoids **1d,e** were separated by column chromatography on silica gel to give **1d-*E***, **1d-*Z***, **1e-*E***, and **1e-*E*** (Scheme 5). Hydrogenation of **1d** and **1e** on Pd/C gave **1(H)d** and **1(H)e**, respectively, in excellent yield (Scheme 5).



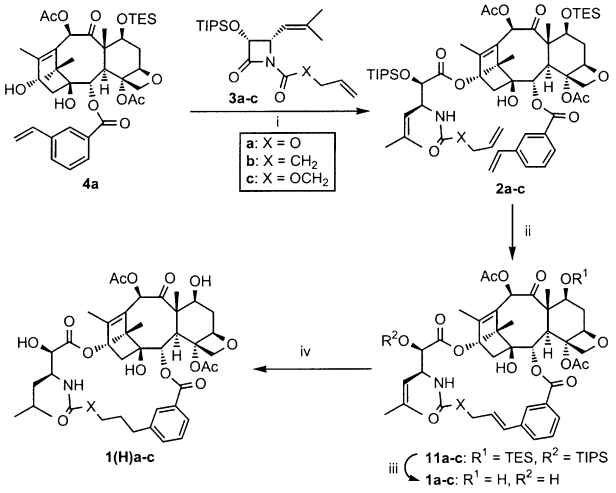
Scheme 1. Retrosynthetic analysis of macrocyclic taxoid **1**.



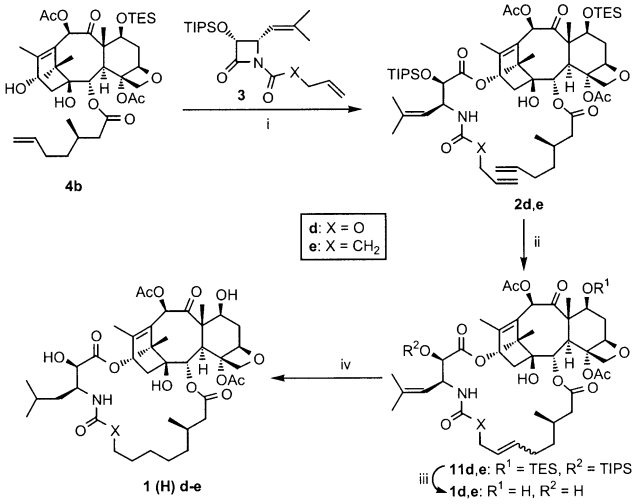
Scheme 2. Synthesis of C-2-modified baccatin III **4**. (i) DIC (3.5 equiv), DMAP, CH_2Cl_2, overnight: **7a**, 54%; **7b**: 89%; (ii) HF-pyridine, pyridine, CH_3CN, overnight; (iii) TESC1 (3.0 equiv), imidazole, (4.0 equiv), DMF, 0 °C to rt, 1 h; **8a**, 95% for two steps; **8b**, 96% for two steps; (iv) LiHMDS (1.1 equiv), AcCl (1.1 equiv), THF, −40 °C, **4a**, 85%; **4b**, 90%.



Scheme 3. Preparation of β-lactam **3**. (i) **10** (2–5 equiv), TEA, (4–6 equiv), DMAP, CH_2Cl_2, 1-2 d, **3a**, 89%; **3b**, 80%; **3c**, 88%.



Scheme 4. Synthesis of macrocyclic taxoids **1a–c** and **1(H)a–c**. (i) LiHMDS (1.5 equiv), **3** (1.5 equiv), THF, −40 °C, 40 min; **2a**, 70%; **2b**, 80%; **2c**, 70%; (ii) $(Cy_3P)_2Ru(=CHPh)Cl_2$ (0.2 equiv), CH_2Cl_2, 2–3 d; **11a**, 87%; **11b**, 70%; **11c**, 96%; (iii) HF-pyridine, pyridine, CH_3CN, overnight; **1a**, 80%; **1b**, 77%; **1c**, 74%; (iv) H_2, Pd/C, EtOAc, overnight, 100%.



Scheme 5. Synthesis of macrocyclic taxoids **1d,e** and **1(H)d,e**. (i) LiHMDS (1.5 equiv), **3** (1.5 equiv), THF, −40 °C, 40 min; **2d**, 93%; **2e**, 77%; (ii) $(Cy_3P)_2Ru(=CHPh)Cl_2$ (0.2 equiv), CH_2Cl_2, 2–3 d; **11d**, 91%, $E/Z=3.5/1$; **11e**, 93%, $E/Z=2/1$; (iii) HF-pyridine, pyridine, CH_3CN, overnight; **1d-*E***, 65%; **1d-*Z***, 18%; **1e-*E***, 56%; **1e-*Z***, 28%; (iv) H_2, Pd/C EtOAc, overnight; **1(H)d**, 95%; **1(H)e**, 99%.

Novel macrocylic taxoids **1a–e** and **1(H)a–e**, thus obtained, were tested for their cytotoxicity against human breast cancer cell lines LCC6-WT and LCC6-MDR (drug-resistant). As Table 1 shows, most of these N-linked series of macrocyclic taxoids exhibit IC_{50} values ranging from micromolar to submicromolar against LCC6-WT cell line. (Note: IC_{50} of paclitaxel-LCC6-WT, 0.0031 μM; LCC6-MDR, 0.346 μM. In particular, **1b**[25] and **1(H)a**[26] exhibit IC_{50} values of 0.09 and 0.1 μM, respectively (entries 2 and 4), which show substantial increase in cytotoxicity as compared to that of the C-linked macrocyclic taxoids.[11,27] Naturally, the cytotoxicity of these N-linked macrocyclic taxoids against a drug-resistant LCC6-MDR cell line is one order of magnitude weaker than that against LCC6-WT. It should be noted, however, the level of resistance observed for these taxoids, 1.35–33 times, is much smaller than that observed for paclitaxel (115 times) against the same pair of LCC6 cell lines. Also, the fact that the *Z*-isomers of **1d** and **1e** possess much better potency than their *E*-isomers (entries 7,8 and 9,10) provides valuable information for further SAR studies.

The development of highly cytotoxic conformationally restricted taxoids, that is macrocyclic taxoids, is of great importance. Those macrocyclic taxoids would provide not only valuable information regarding the bioactive structure of taxane anticancer drugs, but also rationale for design of the next generation anticancer drugs, targeting microtubules, which retain only essential structural feature of paclitaxel or docetaxel but without its structural complexity. Along this line, following our work on the first cytotoxic C-linked macrocyclic taxoids,[10,11] Kingston et al. quite recently reported another C-linked taxoids wherein the C-3′-phenyl and the C-4 acetyl moieties are connected.[29] The advances in the design and synthesis of C-linked and N-linked macrocyclic taxoids together with those in the computational[30,31] and solid-state NMR approaches[32,33] to the identification of tubulin-bound structures of microtubule-stabilizing agents will form a solid basis for the development of the next generation de nove anticancer agents. Further studies along this line are actively underway in these laboratories.

Table 1. Cytotoxicity (IC_{50}, μM)[a] of 3′-N-linked macrocyclic taxoids

Entry	Taxoid	LCC6-WT[b]	LCC6-MDR[c]
1	**1a**	0.30	3.5
2	**1b**	0.09	3.0
3	**1c**	1.9	18
4	**1(H)a**	0.10	1.5
5	**1(H)b**	2.1	21
6	**1(H)c**	7.4	10
7	**1d-*E***	2.7	19
8	**1d-*Z***	0.70	4.0
9	**1e-*E***	2.2	29
10	**1e-*Z***	0.30	9.4
11	**1(H)d**	9.6	19
12	**1(H)e**	2.0	16

[a]The concentration of compound which inhibits 50% of the growth of human tumor cell line after 72 h drug exposure.[28]
[b]LCC6-WT, human breast carcinoma.
[c]LCC6-MDR, MDR1 transduced line.

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References and Notes

1. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
2. Guénard, D.; Guéritte-Vogelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160.
3. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665.
4. Kowalski, R. J.; Giannakakou, P.; Hamel, E. *J. Biol. Chem.* **1997**, *272*, 2534.
5. Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325.
6. Lindel, T.; Jensen, P. R.; Fenical, W.; Long, B. H.; Casazza, A. M.; Carboni, J.; Fairchild, C. R. *J. Am. Chem. Soc.* **1997**, *119*, 8744.
7. ter Haar, E.; Kowalski, R. J.; Hamel, E.; Lin, C. M.; Longley, R. E.; Gunasekera, S. P.; Rosenkranz, H. S.; Day, B. W. *Biochemistry* **1996**, *35*, 243.
8. Corley, D. G.; Herb, R.; Moore, R. E.; Scheuer, P. J.; Paul, V. J. *J. Org. Chem.* **1988**, *53*, 3644.
9. Mooberry, S. L.; Tien, G.; Hernandez, A. H.; Plubrukarn, A.; Davidson, B. S. *Cancer Res.* **1999**, *59*, 653.
10. Ojima, I.; Chakravarty, S.; Inoue, T.; Lin, S.; He, L.; Horwitz, S. B.; Kuduk, S. D.; Danishefsky, S. *J. Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4256.
11. Ojima, I.; Lin, S.; Inuoe, T.; Miller, M. L.; Borella, C. P.; Geng, X.; Walsh, J. J. *J. Am. Chem. Soc.* **2000**, *122*, 5343.
12. Nogales, E.; Wolf, S. G.; Downing, K. H. *Nature* **1998**, *391*, 199.
13. Guéritte-Voegelein, F.; Mangatal, L.; Guénard, D.;

Potier, P.; Guilhem, J.; Cesario, M.; Pascard, C. *Acta Crystallogr*. **1990**, *C46*, 781.
14. Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413.
15. Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C. M.; Brigaud, T. *Tetrahedron* **1992**, *48*, 6985.
16. Ojima, I. *Acc. Chem. Res.* **1995**, *28*, 383, and references cited therein.
17. Holton, R. A.; Biediger, R. J.; Boatman, P. D. In *Taxol®: Science and Applications*; Suffness, M., Ed., CRC: New York, 1995; p 97.
18. Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. In *Taxol®: Science and Applications*; Suffness, M., Ed.; CRC: New York, 1995; p 317.
19. Ojima, I.; Kuduk, S. D.; Chakravarty, S. In *Adv. Med. Chem.*; Maryanoff, B. E., Reitz, A. B., Eds.; JAI: Greenwich, 1998; Vol. 4, p 69.
20. Ojima, I.; Lin, S.; Wang, T. *Curr. Med. Chem.* **1999**, *6*, 927.
21. Ojima, I.; Habus, I.; Zhao, M.; Georg, G. I.; Jayasinghe, R. *J. Org. Chem.* **1991**, *56*, 1681.
22. Ojima, I.; Wang, T.; Miller, M. L.; Lin, S.; Borella, C. P.; Geng, X.; Pera, P.; Bernacki, R. *J. Bioorg. Med. Chem. Lett.* **1999**, *9*, 3423.
23. Nicolaou, K. C.; Renaud, J.; Nantermet, P. G.; Couladouros, E. A.; Guy, R. K.; Wrasidlo, W. *J. Am. Chem. Soc.* **1995**, *117*, 2409.
24. Ojima, I.; Slater, J. S.; Kuduk, S. D.; Takeuchi, C. S.; Gimi, R. H.; Sun, C.-M.; Park, Y. H.; Pera, P.; Veith, J. M.; Bernacki, R. J. *J. Med. Chem.* **1997**, *40*, 267.
25. **1b:** White solid, mp 160–162 °C, $[\alpha]_D^{20}$ −100.0° (*c* 0.16, $CHCl_3$), ^1H NMR (250 MHz, $CDCl_3$) δ 1.12 (s, 3H), 1.25 (m, 6H), 1.6 (s, 3H), 1.7 (s, 3H), 1.8 (s, 3H), 2.14–2.50 (m, 7H), 2.2 (s, 3H), 2.4 (s, 3H), 3.76 (d, J = 7.4 Hz, 1H), 4.28 (dd, J = 21.3, 8.4 Hz, 1H), 4.44 (b, 1H), 4.94 (m, 2H), 5.29–5.36 (m, 3H), 5.64 (d, J = 7.4 Hz, 1H), 5.74 (d, J = 7.2 Hz, 1H), 6.25–6.28 (m, 3H), 6.45 (d, J = 15.9 Hz, 1H), 7.31–7.48 (m, 2H), 7.87 (d, J = 7.5 Hz, 1H), 8.16, (s, 1H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 5.3, 9.6, 10.0, 14.0, 16.3, 18.2, 18.6, 18.8, 21.3, 22.6, 23.0, 24.8, 25.2, 27.4, 30.8, 32.3, 38.5, 40.8, 45.7, 53.8, 67.4, 67.9, 68.0, 70.8, 76.0, 76.2, 80.0, 116.1, 120.3, 124.5, 124.5, 125.0, 125.1, 125.8, 128.3, 128.7, 133.3, 133.7, 137.7, 162.5, 166.5, 166.8, 167.1, 168.4, 199.2. HRMS (FAB, DCM/NBA/NaCl) *m*/*z* calcd for $C_{43}H_{53}NO_{14}Na^+$ 832.3364, found 832.3369 (Δ = −0.6 ppm).
26. **1(H)a:** mp 182–184 °C; $[\alpha]_D^{20}$ −33.3° (*c* 0.12, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.86 (m, 6H), 1.13 (s, 3H), 1.25 (m, 5H), 1.29 (s, 3H), 1.59 (s, 3H), 1.69 (s, 3H), 1.78 (s, 3H), 2.02 (m, 2H), 2.24, (s, 3H), 2.30 (m, 2H), 2.37 (s, 3H), 2.44 (s, 3H), 2.80 (m, 1H), 3.80 (d, J = 7.7 Hz, 1H), 4.24 (m, 2H), 4.47 (b, 1H), 4.81 (b, 1H), 4.90 (d, J = 9.3 Hz 1H), 5.14 (b, 1H), 5.35 (d, J = 5.4 Hz, 1H), 5.63 (d, J = 7.7 Hz, 1H), 6.26, (m, 2H), 7.38 (b, 2H), 7.89 (s, 1H), 8.17 (s, 1H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 5.4, 9.6, 10.1, 14.2, 16.3, 18.2, 18.4, 21.3, 22.4, 24.6, 24.8, 25.2, 26.7, 27.4, 30.8, 30.8, 40.9, 47.2, 53.2, 53.8, 58.3, 67.4, 67.8, 70.9, 80.1, 115.0, 123.77, 124.4, 124.9, 126.0, 129.6, 135.5, 137.8, 149.2, 162.0, 165.8, 166.8, 170.2, 199.1. HRMS (FAB, DCM/NBA/NaCl) *m*/*z* calcd for $C_{42}H_{55}NO_{15}Na^+$ 832.3156, found 832.3131 (Δ = 3.1 ppm).
27. Boge, T. C.; Wu, Z.-J.; Himes, R. H.; Vander, D. G.; Georg, G. I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3047.
28. Skehan, P.; Streng, R.; Scudierok, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Nat. Cancer Inst.* **1990**, *82*, 1107.
29. Metaferia, B. B.; Hoch, J.; Glass, T. E.; Bane, S. L.; Chatterjee, S. K.; Snyder, J. P.; Lakdawala, A.; Cornett, B.; Kingston, D. G. I. *Org. Lett.* **2001**, *3*, 2461.
30. Snyder, J. P.; Nettles, J. H.; Cornett, B.; Downing, K. H.; Nogales, E. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5312.
31. Geney, R., Simmerling, C. L., Ojima, I. *Abstracts*, 222nd American Chemical Society National Meeting, 2001; MEDI65.
32. Li, Y.; Poliks, B.; Cegelski, L.; Poliks, M.; Cryczynski, A.; Piszcek, G.; Jagtap, P. G.; Studelska, D. R.; Kingston, D. G. I.; Schaefer, J.; Bane, S. *Biochemistry* **2000**, *39*, 281.
33. Ojima, I., Inoue, T., Slater, J. C., Lin, S., Kuduk, S. C., Chakravarty, S., Walsh, J. J., Gilchrist, L., McDermott, A. E., Cresteil, T., Monsarrat, B., Pera, P., Bernacki, R. J. In *Asymmetric Fluoroorganic Chemistry: Synthesis, Application, and Future Directions*, Ramachandran, Ed.; ACS Symp. Ser. 746; American Chemical Society: Washington, DC, 1999; p 158.