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Synthesis and Biological Evaluation of Novel 9-Functional Heterocyclic Coupled 7-Deoxy-9-Dihydropaclitaxel Analogue

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Abstract—Novel 9-functional heterocyclic coupled 7-deoxy-9-dihydropaclitaxel analogues **17** and **22**–**24** synthesized from a natural taxoid 5-cinnamoyltriacetyltaxicin-I (**3**) and their biological evaluation in tubulin assembly activity and cytotoxicity in vitro against several human tumor cell lines are first presented. The biologically tested results show that **17, 22** and **23** are inactive in tubulin assembly assay and have no more remarkable cytotoxicities against human tumor cell lines SK-0V3, WIDR and MCF-7, though **22** and **23** exhibit more potent cytotoxicity against human liver cancer and human esophagus cancer cell lines (BEL-7402 and ECa-109) than paclitaxel.

The diterpenoid paclitaxel (Taxol®, **1**), originally isolated from the Pacific yew tree *Taxus brevofolia* in 1971,[1] exhibits remarkably high cytotoxicity and strong antitumor activity against different tumors resistantly treated by existing anticancer drugs.[2] It has been approved for treatment of advanced ovarian and breast cancers,[3,4] and it is currently in clinical trials for treatment of lung, skin, and head and neck cancers with encouraging results.[5] Since its discovery in the 1960s, structural complexity, important biological activity and novel mechanism of action[6,7] of pacitaxel have stimulated extensive chemical, biological and medicinal research.[8,9] Up to date, numerous analogues have been prepared among many studies of structural modifications of paclitaxel by contractions and changes to A-ring,[10,11] B-ring,[12,13] C-ring,[14,15] D-ring,[16,17] and the side-chain,[18] which have led to the discovery of the new and potent bioactive taxoid docetaxel[19] (Taxotere® **2**).

A large number of the structure–activity relationships (SAR) studies of paclitaxel have led to the general conclusions that the function groups at C-7, C-9 and C-10 in the 'northern hemisphere' have little effect on bioactivity, while benzoyloxy and acetoxy at C-2 and C-4 respectively plus the oxetane ring in the 'southern hemisphere' and the side chain at C-13 are essential for biological activity.[8,9,20,21] However, only a few modifications of C-9 such as dihydrogenation and decarboxylation have been reported.[22] Recently, further chemical and SAR studies of C-9 have few appeared in the literature. In addition, twin drugs combining two biologically active components into a single molecule have been reported in numerous domains of medicinal chemistry.[23] Thus, according to the previous SAR studies which show 7-dehydroxylation having no significant effect on the activity of pacitaxel, we expected to explore the possibility of new taxoids combined with the functional heterocyclic such as AZT, which is the first therapeutic drug for AIDS in clinical medicine, possessing potent cytotoxicity or other biological activity.

Recently, the semisyntheses of 7-deoxypaclitaxel analogues have been reported,[24–27] but all investigations have been carried out by using taxine B and isotaxine B[28] as starting materials. So far attention has never been paid to 5-cinnamoyltriacetyltaxicin I (**3**),[29] the major taxoid isolated in abundant yield from the needles of different *Taxus* species, such as Japanese yew *T. cuspidata,* as a precursor for the synthesis of either paclitaxel or its analogues. In this communication, we report our efforts

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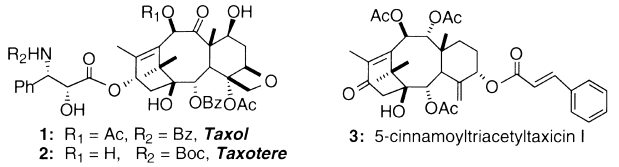


Chart 1.

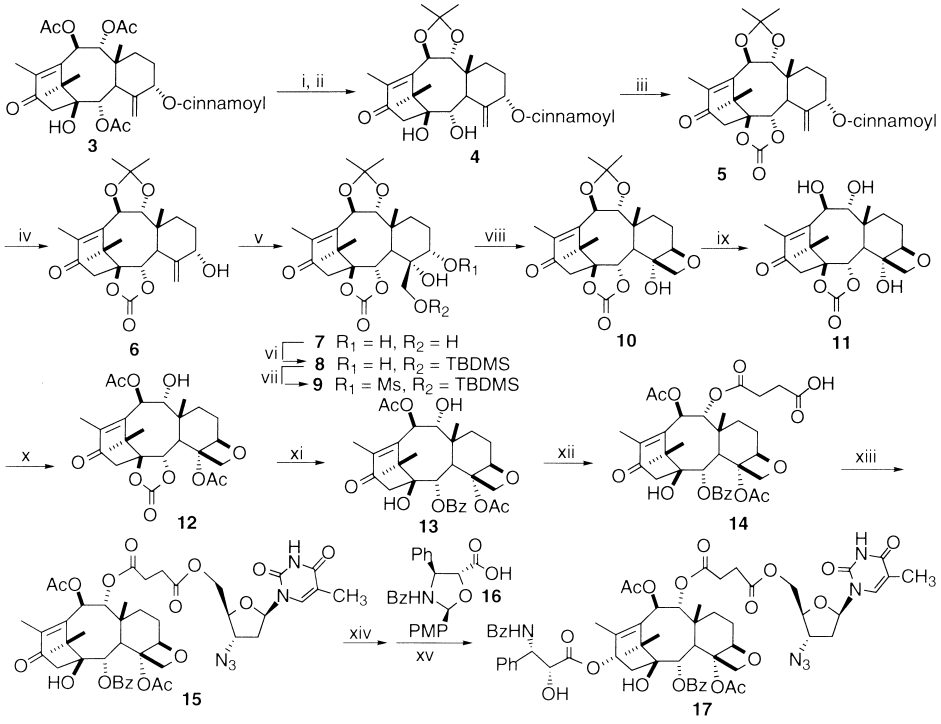
on the synthesis and biological evaluation of several new 7-deoxy-9-dihydropaclitaxel analogues **17** and **22**–**24** from 5-cinnamoyltriacetyltaxicin I (**3**).

As depicted in Scheme 1, a natural taxoid **3** underwent deacetylation and selective protection of 9,10-dihydroxy with acetone catalyzed by $CuSO_4$[24] to give **4** in 73% yield. Further protection of 1,2-dihydroxy with triphosgene followed by hydrolysis of the cinnamate ester using hydroxylamine[30] led to 5-hydroxy–taxoid **6**. Construction of the oxetane ring from **6** was achieved following the previously established methods[24–27] leading to **10.** We expected that the isopropylidene group could be removed independently from the 1,2-cyclic carbonate and oxetane ring. This attempt was accomplished by treatment of **10** with a mixture of HOAc:H_2O:THF (v/v 4:2:l) at 40 °C to afford 4, 9, 10-trihydroxy taxoid **11** in 71% yield. In fact, this selective acid hydrolysis in this taxoid series has already been reported.[24] Acetylation of **11** by means of Ac_2O/DMAP gave 4- and 10-diacetylated 12,[31] which subsequently was treated with phenyllithium in THF at −78 °C to furnish compound **13**.

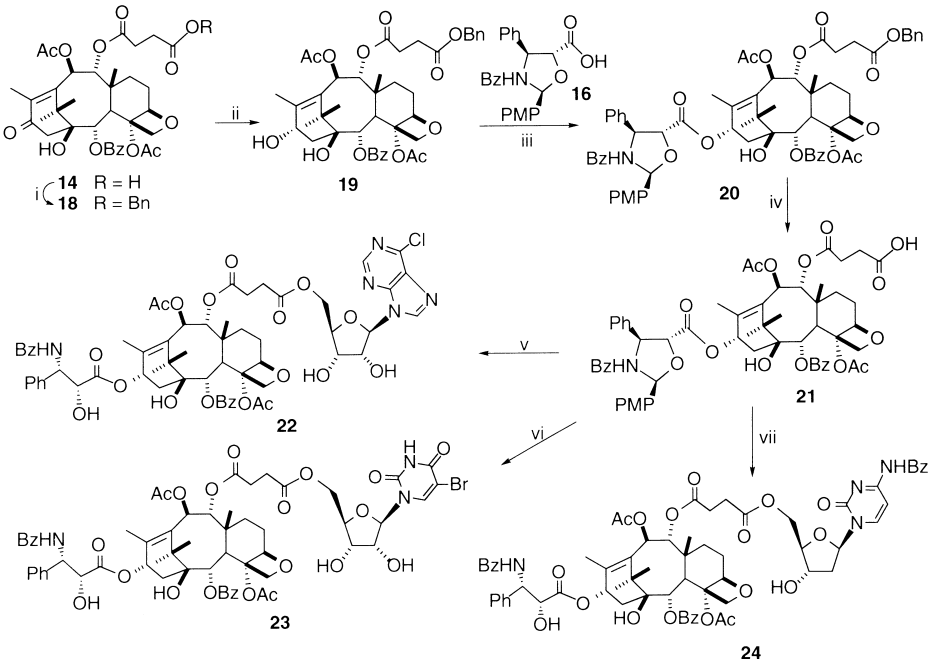
In order to achieve the combination of taxoid **13** with biologically active compound AZT, the succinyl group was introduced as a bridge to connect the above two molecules. Compound **13** was treated with an excess of succinic anhydride to provide the succinate **14** as the only reaction product uneventfully, subsequently which was reacted with AZT in the presence of DMAP to give **15** in 85% yield. Reduction of **15** and subsequent esterification with (*2S*, *4S*, 5*R*)-3-benzoyl-2-(*p*-methoxyphenyl)-4-phenyl-1,3-oxazolidine-5-carboxylic acid **16** and acid hydrolysis afforded the desired product **17**[32] in 51% all yield from **15** according to the developed established methods.[33]

The mechanism of action studies of paclitaxel indicate that the anticancer activity is believed to be mediated by a combination of its primary action on microtubule assembly and secondarily by inhibiting DNA synthesis and promoting DNA fragmentation of the cell.[28] These actions are found to be related to the nucleotide which is a basic unit in the formation of nucleic acids DNA and RNA. In order to explore the possibility of new taxoids which are expected to have highly biological activities, three novel 9-functional nucleotide coupled 7-deoxy-9-dihydropaclitaxel analogues **22**–**24** were synthesized by introduction of functional nucleotides (Scheme 2).

After esterification of succinyl taxoid **14** by benzyl alcohol which gave a corresponding ester **18**, subsequently this was reduced to give 13α-hydroxy taxoid **19**



Scheme 1. Reagents and conditions: (i) $NaOCH_3$, CH_3OH/CH_2Cl_2, 0 °C, 86%; (ii) Acetone, CuSO, 73%; (iii) $(Cl_3CO)_2CO$ (2 eq), CH_2Cl_2:Pyridine (2:1), 0 °C, 92%; (iv) $NH_2OH.HCl$, NaOAc, $EtOH/H_2O$, reflux, 65%; (v) OsO_4 NMO, THF:H_2O (2:1). rt, 86%; (vi) TBDMSCl, imidazole, DMF, rt, 95%; (vii) MsCl, pyridine, 81%; (viii) a: TBAF, THF, b: $Bu_4N^+{}^-OAc$, butanone, reflux, 75%; (ix) HOAc:H_2O:THF (v/v 4:2:1), 40 °C, 71%; (x) Ac_2O, DMAP, 0 °C to rt, 76%; (xi) PhLi, THF, −78 °C, 84%; (xii) Succinic anhydride, DMAP, DMF, 85 °C. 48 h, 69%; (xiii) AZT, DCC, DMAP, toluene, 85%; (xiv) $NaBH_4$, CH_3OH. rt, 78%; (xv) a: DCC, DMAP, toluene; b: 0.1 N HCl, CH_3OH, 65%.



Scheme 2. Reagents and conditions: (i) $PhCH_2OH$, DCC, DMAP, toluene, 90%; (ii) $NaBH_4$, CH_3OH, rt, 83%; (iii) DCC, DMAP, toluene, 86%: (iv) H_2, Pd/C (10%), EtOAc, rt, 73%; (v) a: 6-Chloropurine riboside, DCC, DMAP, toluene; b: TFA, H_2O, rt, 73% from **21;** (vi) a: (−)-5-Bromouridine, DCC, DMAP, toluene; b: TFA, H_2O, rt, 69% from **21**; (vii) a: N^4-Benzoyl-2′-deoxycytidine, DCC, DMAP, toluene; b: TFA, H_2O, rt, 75% from **21**.

followed by treatment with carboxylic acid **16** which afforded the reaction product **20**. Removal of the benzyl group by hydrogenation in the presence of Pd (10% on activated carbon) gave the C-13 side-chain protected acid **21** in 73% yield. Combination of the acid **21** with 6-chloropurine riboside using DCC and a catalytic amount of DMAP in toluene, followed by acid hydrolysis with trifluoroacetic acid and water, afforded the desired product **22** in 73% yield. A similar combination of the acid **21** with (−)-5-bromouridine and N^4-benzoyl-2′-deoxycytidine and followed by acid hydrolysis gave the compounds **23** and **24** in 69 and 75% yields, respectively.

Biological activities of the novel 7-deoxy-9-dihydropaclitaxel analogues **17** and **22**–**24** were evaluated in two assay systems, i.e. in vitro cytotoxicity against five human tumor cell lines (SK-OV3, WIDR, MCF-7, BEL-7402, ECa-109) and microtubule assembly activity.[34] The results are presented in Table 1. Compared to paclitaxel, **17**, **22** and **23** showed a marked decrease of tubulin assembly activity and **24** remained at half of the tubulin assembly activity of paclitaxel in our experimental conditions. This implies that the functional group at C-9 may be one effective factor of the microtubulin binding site beside another two key microtubulin binding regions involving the C-13 ester side chain and the oxetane ring of paclitaxel.[35] In cytotoxicity assay **17** and **22**–**24** showed less cytotoxicity against three human cancer cell lines (SK-OV3, WIDR and MCF-7) than paclitaxel, though both **22** and **23** showed more potent cytotoxicity against human liver cancer (BEL-7402) and human esophagus cancer (ECa-109) cell lines than paclitaxel. What reasons result in this inconsistency between tubulin polymerisation activity and cytotoxicity of **22** and **23** against BEL-7402 and ECa-109 is as yet unclear, but it might be explained either by the success of **22** and **23** to gain access to the

Table 1. Biological assays in microtubule assembly activity and cytotoxicity of **17** and **22**–**24**

Compound	Cytotoxicity against tumor cell (Ed_{50}/ED_{50})[a,b,c]					Microtubule assembly assay (ED_{50} μM)[d]
	SK-OV3	WIDR	MCF-7	BEL-7402	ECa-109	—
17	3.41	2.62	3.13	7.32	3.54	>20
22	2.24	2.16	3.68	0.24	0.18	>15
23	4.06	3.87	2.64	0.13	0.16	>15
24	1.98	2.43	1.63	1.45	1.03	0.9
Paclitaxel	1.00	1.00	1.00	1.00	1.00	0.4

[a]ED_{50} is the concentration which produces 50% inhibition of proliferation after 72 h of incubation.
[b]Ratio of ED_{50} relative to paclitaxel is 1.00.
[c]SK-OV3: human ovarian cancer; WIDR: human colon cancer MCF-7: human breast cancer; BEL-7402: human liver cancer; ECa-109: human esophagus cancer.
[d]ED_{50} is the concentration which causes polymerization of 50% of the tubulin present at 37 °C.

cells or by some biotransformations of **22** and **23** to an active metabolite. These compounds are likely to induce cell death via apoptosis, a process characterized by cytoskeletal changes, chromatin condensation, and genomic DNA fragmentation as paclitaxel.[36] In fact, this difference between tubulin polymerisation activity and cytotoxicity in paclitaxel analogues has already been reported[35,37].

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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. Suffness, M. *Ann. Rep. Med. Chem.* **1993**, *28*, 305.
3. Rowinsky, E. K.; Cazenave, L. A.; Donehower, R. C. *J. Natl. Cancer Inst.* **1990**, *82*, 1247.
4. Mcguire, W. P.; Rowinsky, E. K.; Rosenshein, N. B.; Grumbine, F. C.; Ettinger, D. S.; Armstrong, D. K.; Donehower, R. C. *Ann. Intern. Med.* **1989**, *111*, 273.
5. Holmes, F. A.; Kudelka, A. P.; Kavanagh, J. J.; Huber, M. H.; Ajani, J. A.; Valero, V. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M. Eds.; ACS Symposium Series 583, American Chemical Society, Washington, DC, 1995, pp 31–57.
6. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665.
7. Manfredi, J. J.; Horwitz, S. B. *Pharmacol. Ther.* **1984**, *25*, 83.
8. Nicolaou, K. C.; Dai, W.-M.; Guy, R. K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 15.
9. 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 Press, Inc., Boca Raton, FL, USA, 1995, pp 317–375.
10. Chen, S. H.; Huang, S.; Wei, J.; Farina, V. *J. Org. Chem.* **1993**, *58*, 4520.
11. Yuan, H.; Kingston, D. I. *Tetrahedron* **1999**, *55*, 9089.
12. Guéritte-Voegelein, F.; Guénard, D.: Dudois, J.; Wahl, A.; Marder, R.; Muller, R.; Lund, M.; Bricard, L.; Potier, P. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M. Eds.; ACS Symposium Series 583, American Chemical Society, Washington, DC, 1995, pp 189–202.
13. Chen, S. H.; Farina, V. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M. Eds.; ACS Symposium Series 583, American Chemical Society, Washington, DC, 1995, pp 247–261.
14. Liang, X.; Kingston, D. G. I.; Long, B. H.; Farichild, C. A.; Johnston, K. A. *Tetrahedron* **1997**, *53*, 3441.
15. Wittman, M. D.; Alstadt, T. J.; Kadow, J. F.; Vyas, D. M.; Johnson, K.; Farichild, C.; Long, B. *Tetrahedron Lett.* **1999**, *40*, 4943.
16. Marder-Karsenti, R.; Dubois, J.; Bricard, L.; Guénard, D.; Guéritte-Voegelein, F. *J. Org. Chem.* **1997**, *62*, 6631.
17. 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.
18. Commerçon, A.; Bourzat, J. D.; Didier, E.; Lavelle, F. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M. Eds.; ACS Symposium Series 583, American Chemical Society, Washington, DC, 1995, pp 233–246.
19. Guénard, D.; Guéritte-Voegelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160.
20. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M. Eds.; ACS Symposium Series 583, American Chemical Society, Washington, DC, 1995, pp 203–216.
21. In *The Chemistiy and Pharmacology of Taxol and Its Derivatives*; Farina, V. Ed.; Elsevier, Amsterdam, The Netherlands, 1995, pp 165–254.
22. In *Taxane AnticancerAgents: Basic Science and Current Status*; Georg, G. I.; Chen, T. T.; Ojima, I.: Vyas, D. M. Eds.; ACS Symposium Series 583, American Chemical Society, Washington, DC, 1995, pp 276–287.
23. Bourgignon, J. J. In *The Practice of Medicinal Chemistry*; Wermuth, C. G. Ed.; Academic Press, 1996. pp 261–293.
24. Wiegerinck, P. H. G.; Fluks, L.; Hammink, J. B.; Mulders, S. J. E.; Groot, F. M. H.; Van Rozendaal, L. M.; Scheeren, H. W. *J. Org. Chem.* **1996**, *61*, 7092.
25. Poujol, H.; Ahond, A.; Mourabit, A.; Chiaroni, A.; Poupat, C.; Riche, C.; Potier, P. *Tetrahedron* **1997**, *53*, 5169.
26. Poujol, H.; Mourabit, A.; Ahond, A.; Poupat, C.; Potier, P. *Tetrahedron* **1997**, *53*, 12575.
27. Matovic, R. and Saicic, R. N. *J. Chem. Soc., Chem. Commun.*, **1998**, 1745
28. Graf, E., Weinandy, S., Koch, B. and Breitmaier, E. *Liebigs Ann. Chem.* **1986**, 1147
29. Appendino, O.; Gariboldi, P.; Pisetta, A.; Bombardelli, E.; Gabetta, B. *Phytochemistry* **1992**, *31*, 4253.
30. Cheng, Q.; Oritani, T.; Horiguchi, T. *Tetrahedron* **1999**, *55*, 12099.
31. In addition to **12**, the 10-hydroxy-2-benzoyl-2, 10, 13-trideacetyl-7-deacetoxy-13-oxo baccatine IV was obtained in a small amounts.
32. All products have been elucidated by spectroscopic data including 2D-NMR and the selected data of a representative **17** as follows: $[\alpha]_D^{23}$ +13.4° (c 0.02, CH_3OH), ^1H NMR (500 MHz, $CDCl_3$) δ 8.13 (dd, 2H, J=8.3, 1.3 Hz, BzO), 7.81 (d, 1H, J=1.0 Hz, =CH), 7.74 (dd, 2H, J=8.5, 1.2 Hz, BzN), 7.62 (t, 1H, J=7.6 Hz, BzO), 7.50 (m, 2H, BzO), 7.49–7.48 (m, 3H, BzN, Ph), 7.41 (m, 4H, BzN, Ph), 7.30 (m, 1H, Ph), 6.97 (d, 1H, J=9.0 Hz, NH), 6.19 (t, 1H, J=8.6 Hz, H-13), 6.17 (t, 1H, J=6.5 Hz, CH), 6.10 (d, 1H, J=10.2 Hz, H-10), 5.90 (d, 1H, J=10.3 Hz, H-9), 5.84 (dd, 1H, J=9.0, 3.0 Hz, H-3′), 5.70 (d, 1H, J=7.0 Hz, H-2), 4.95 (d, 1H, J=8.7 Hz, H-5), 4.56 (dd, 1H, J=4.0, 3.0 Hz, H-2′), 4.36 (td, lH, J=6.5, 5.0 Hz, N_3CH), 4.33 (d, 1H, J=8.4 Hz, H-20), 4.18 (d, 1H, J=8.4 Hz, H-20), 3.90 (td, 1H, J=5.0, 3.5 Hz, CH), 3.83 (dd, 1H, J=12.5, 3.0 Hz, OCH_2), 3.74 (dd, 1H, J=12.5, 3.5 Hz, OCH_2), 3.52 (d, 1H, J=4.0 Hz, OH-2′), 2.70 (m, 4H, $2CH_2$), 2.38 (m, 2H, CH_2), 2.36 (dd, 1H, J=15.0, 8.5 Hz, H-14), 2.27 (s, 3H, Ac-4), 2.23 (s, 3H, Ac-10), 2.21 (m, 1H, H-6), 2.18 (dd, 1H, J=15.0, 8.5 Hz, H-14), 1.94 (m, 1H, H-6), 1.88 (d, 3H, J=1.0 Hz, CH_3), 1.87 (s, 3H, H_3-18), 1.84 (m, 1H, H-7), 1.63 (s, 3H, H_3-19), 1.59 (dd, 1H, J=14.5, 4.8 Hz, H-7), 1.32 (s, 3H, H_3-16), 1.11 (s, 3H, H_3-17). ^{13}C NMR (125 MHz) δ 172.6 (s, 1′), 171.3 (s, Ac-10), 170.4 (s, Ac-4), 170.1 (s), 169.9 (s), 167.3 (s, BzO), 167.0 (s, BzN), 166.4 (s), 152.3 (s), 142.3 (s, 12), 138.1 (s), 138.0 (s, Ph), 133.7 (d, Ph), 133.6 (s, Ph), 133.5 (s, 11), 132.1 (d, Ph), 130.3 (d,Ph), 129.1 (s, Ph), 129.0 (d, Ph), 128.7 (d, Ph), 128.6 (d, Ph), 128.4 (d, Ph), 127.1 (d, Ph), 127.0 (d, Ph), 111.6 (d), 86.1 (d), 86.0 (d), 85.4 (d, 5), 82. 1 (s, 4), 78.9 (s, 1), 76.4 (t, 20), 76.3 (d, 9), 74.7 (d, 2), 73.2 (d, 10), 73. 1 (d, 2′), 72.4 (d, 13), 62.4 (t), 61.6 (d), 55.2 (d, 3′), 43.6 (s, 8), 42.9 (s, 15), 42.2 (d, 3), 38.3 (t), 35.8 (t, 14), 28.8 (t), 28.5 (t), 27.4 (t, 6), 27.1 (t, 7), 26.5 (q, 17), 22.4 (q, Ac-4), 21.5 (q, 16), 20.6 (q, Ac-10), 17.5 (q, 19), 14.7 (q, 18), 12.5 (q). HRMS-FAB (m/z calcd for $C_{46}H_{68}O_{19}N_6Na$ $(M+Na)^+$ 1031.4432, found 1031.4423.

33. Mukaiyama, T.; Shiina, I.; Iwadare, H.; Saitoh, M.; Nishimura, T.; Ohkawa, N.; Sakoh, H.; Nishimura, K.; Tani, Y.; Hasegawa, M.; Yamada, K.; Saitoh, K. *Chem. Eur*. **1999**, *5*, 121and references cited therein.
34. All, S. M.; Hoemann, M. Z.; Aubé, J.; Mistcher, L. A.; Georg, G. I.; Mccall, R.; Jayasinghe, L. R. *J. Med. Chem*. **1995**, *38*, 2821. For review see ref 9.
35. Samaranaye, G.; Magri, N. F.; Jitrangsri, C.; Kingston, D. G. I. *J. Org. Chem*. **1991**, *56*, 5114.
36. Bhalla, K.; Ibrado, A. M.; Tourkina, E.; Tang, C.; Mahoney, M. E.; Huang, Y. *Leukemia* (Baltimore) **1993**, *7*, 563.
37. Appendino, O.; Belloro, E.; Jakupovic, S.; Danieli, B.; Jakupovic, J.; Bombardelli, E. *Tetrahedron* **1999**, *55*, 6567 and see refs 26 and 35.