Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 419–424

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ON THE RELATIONSHIP OF OSW-1 TO THE CEPHALOSTATINS

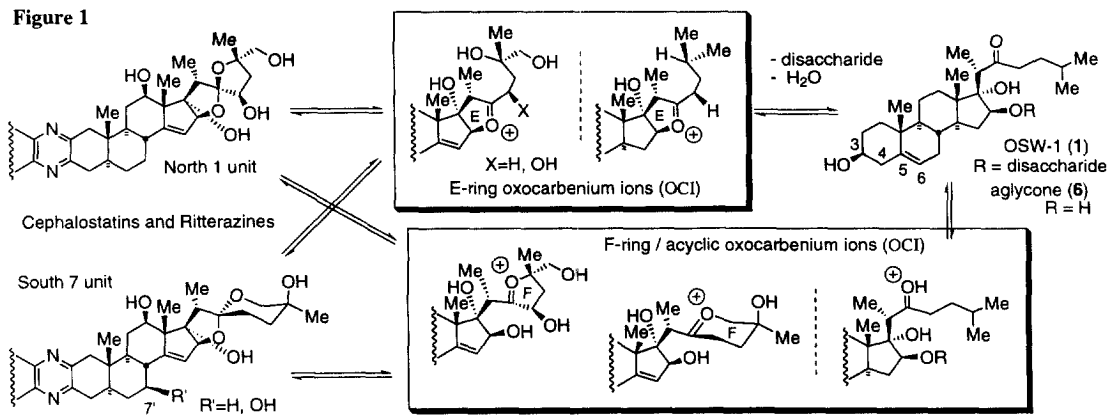
Chuangxing Guo, Thomas G. LaCour* and P. L. Fuchs*
Department of Chemistry, Purdue University, West Lafayette, IN 47907, U.S.A.

Received 16 October 1998; accepted 18 December 1998

Abstract: Antineoplastic bis-steroidal (cephalostatin-type) analogues of the saponin OSW-1 were produced from a dihydroaglycone of OSW-1. The key aglycone **6H** was obtained from 5α-androstan-3β-ol-17-one in 8 steps (38% yield). The SAR of the aglycones, intermediates, and hybrid analogues provide insights regarding the proposed common role of C22-oxocarbenium ions in the bioactivity of both OSW-1 and cephalostatins

The cephalostatins[1] and ritterazines[2] are antineoplastic bis-steroidal natural products (sub-nanomolar activity) from separate marine phyla, while saponin OSW-1 (**1**) belongs to a plant-derived (*Ornithogalum saundersiae*) family of potent mono-steroidal antitumor agents (GI_{50} 0.78 nM for **1**).[3] The cytotoxicity profile of **1** was found to be surprisingly similar to that of the cephalostatins, with correlation coefficients of 0.60–0.83, which appears to imply a related mechanism of action.[3] Previously, an E-ring oxocarbenium ion (OCI) was proposed as a common active intermediate[4] (Fig. 1). This would necessitate loss of the sugars and water from **1** before putative covalent binding. Another possibility, analogous to F-ring ions in the spiroketal series,[5] is an acyclic OCI which could be accessed without prior hydrolysis of the glycoside linkage. To interrogate such ions as potential pharmacophores, we sought cephalostatin-type analogues incorporating the OSW-1 aglycone.

Figure 1

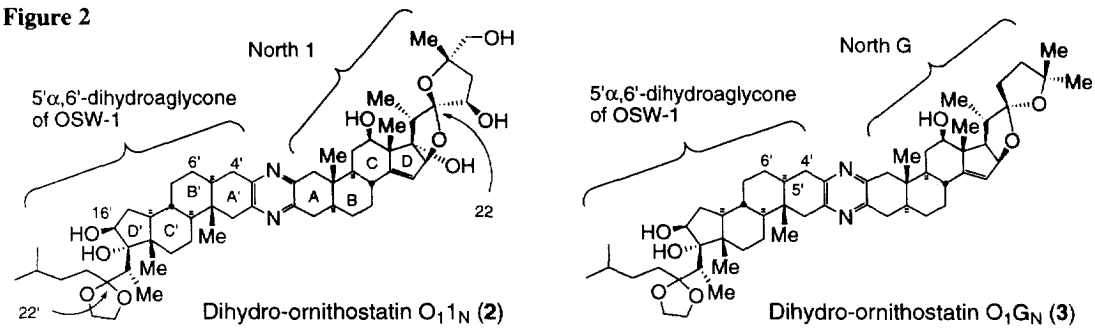


Design and Synthesis of Analogues

Since the "interphylal" hybrid pyrazine ritterostatin G_N1_N (not shown) composed of the upper hemispheres of cephalostatin 1 (North 1)[6,7] and ritterazine G (North G)[6] was found to be a potent antitumor agent (GI_{50} 14 nM),[6] the cytotoxicities of the proposed "interkingdom" hybrids were expected to provide significant insights.

0960-894X/99/$ - see front matter
PII: S0960-894X(98)00743-4

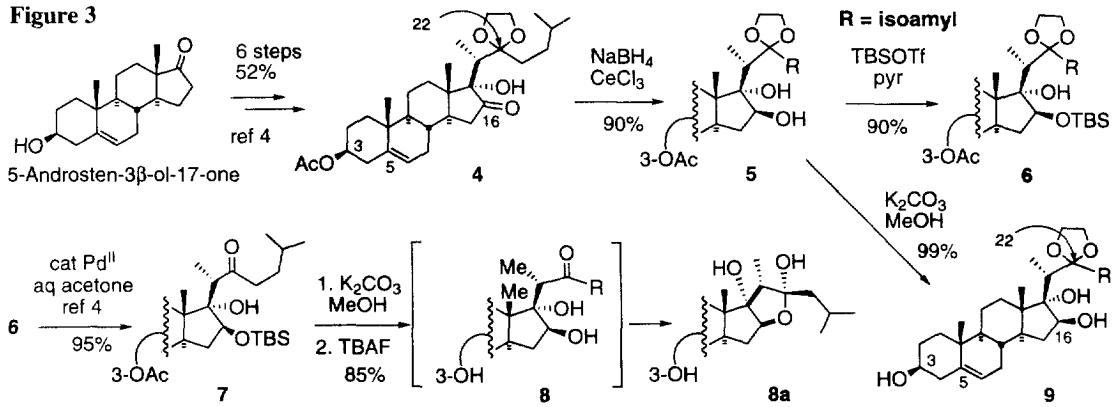
Figure 2



The North 1 and North G subunits were therefore each chosen as a coupling partner for the OSW-1 aglycone in construction of the analogues dihydro-ornithostatin $O_1 1_N$ (**2**)[8] and dihydro-ornithozine $O_1 G_N$ (**3**)[8] (Fig. 2).

The C22 ketal form of the aglycone partner was chosen for these analogues in preference to the free ketone because the natural aglycone **8** (which adopted the hemi-ketal form **8a** as expected, Fig. 3)[9] was found to be less active than its ketal **9** (*vide infra*).[10] Both compounds were synthesized from the known intermediate **4**.[4] We

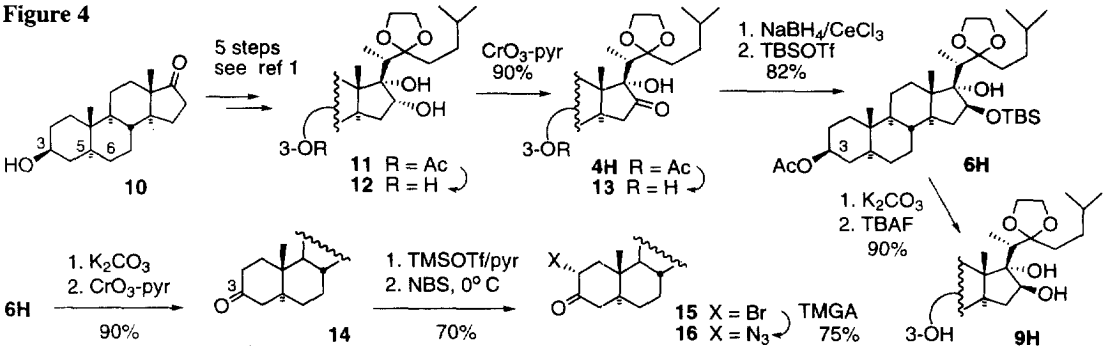
Figure 3



elected to prepare the hybrid cephalostatin analogues with the saturated aglycone ketal **9H** (Fig. 4). This fusion maintains a pentacyclic pyrazine core structure (B-B' rings, Fig. 2) identical to that of the cephalostatins and ritterazines. We hoped that the A/B ring flattening caused by pyrazine fusion would be functionally similar to that induced by the Δ^5 function present in OSW-1. Moreover, when C3 is a ketone, the natural 5,6-olefin is prone to isomerize into conjugation as the A-ring enone.

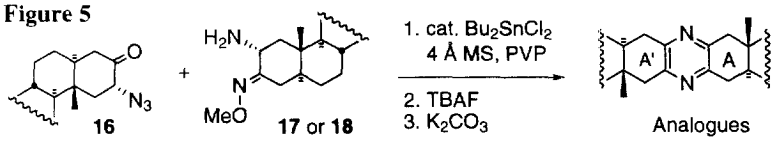
The saturated derivative **6H** (Fig. 4) was prepared in 38% overall yield in 8 steps from 5α-androstan-3β-ol-17-one (**10**) via the same synthetic sequence utilized for **6**.[4] For SAR studies, hydroxyl deprotections in **11**, **4H**, and **6H** gave **12**, **13**, and **9H**, respectively. Generation of the coupling partner **16** began with C3 hydrolysis and oxidation of **6H** to afford 16,22-protected 3-ketone **14** in excellent yield. Attempted bromination of **14** in the usual manner[6,11] using phenyltrimethylammonium perbromide (PTAB) in THF produced bromoketone **15** in less than 20% yield, presumably due to HBr cleavage of the ketal. Fortunately, NBS bromination[12] of the TMS silyl enol ether (not shown) gave the desired bromide **15** in 70% yield. Reaction with tetramethylguanidinium azide (TMGA) in nitromethane[11] smoothly afforded **16**.

Figure 4



Azidoketone **16** condensed with the protected aminomethoximes of North 1 (**17**)[13] and North G (**18**)[6] via our general protocol[13] to smoothly furnish the desired bis-steroidal, cephalostatin-type undecacyclic pyrazines, which upon deprotection gave the analogue dihydro-ornithostatins **2** and **3** (Fig. 5).

Figure 5



Coupling and deprotection

Partners	Pyrazine	Analogue
16 and **17**	71%	**2** (71%)
16 and **18**	61%	**3** (76%)

Antitumor Activity Results and Analysis

Cytotoxicity data[14] for the aglycones, certain intermediates, and the analogues is summarized in Table 2. As previously mentioned, the aglycone **8a** was generally less active than its ketal **9**. At first glance, this result appears to disfavor an E-ring oxocarbenium ion (E-OCI) as an intermediate for covalent binding, since both compounds would give the same E-ring ion (**8a**$^+$, Fig. 6). If E-OCI **8a**$^+$ were the active intermediate, **8a** might reasonably be expected to access such an E-OCI more readily and show higher activity than **9**. On the other hand, binding by the oxocarbenium ion may depend upon the timing of its formation. The hemiketal in **8a** could prematurely dehydrate via **8a**$^+$ to form enol ether **8b**[15] and undergo Ferrier-type elimination to produce an aromatized E-ring furan **8c** such as that previously observed in this series.[4] Either of these (**8b/c**) might be responsible for the observed reduction in cytotoxicity. By contrast, both **1** and **9** *must* proceed through an intermediate acyclic oxocarbenium ion in order to form an E-OCI, as they are "protected" (16-OGly and 22-ketal, respectively) from direct production of **8a**$^+$ by several intervening chemical steps, including requisite loss of the disaccharide or diol moieties.

Figure 6

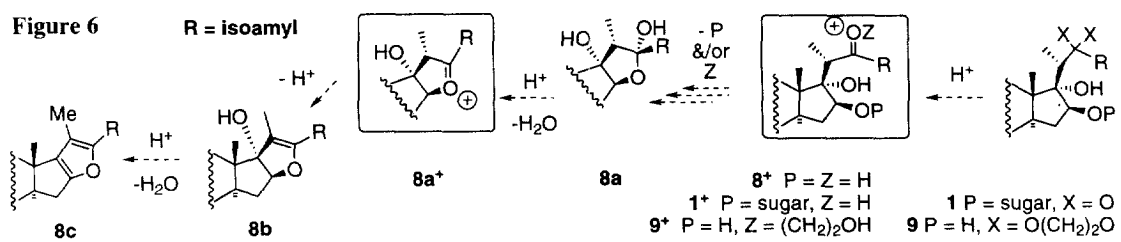
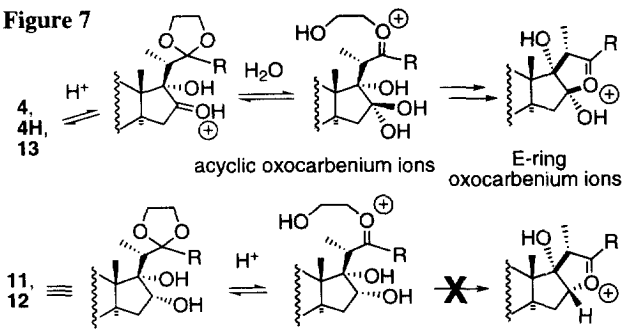


Table 1. Cytotoxicities against representative cell lines in the Purdue Cell Culture Laboratory screen (ED_{50} μM).[14]

Compound	A-549	MCF-7R	HT-29	A-498	PC-3	PACA-2	MCF-7	MCF-7ADR
Adriamycin	6.2×10^{-3}	0.60	3.7×10^{-2}	3.5×10^{-3}	5.7×10^{-2}	6.3×10^{-3}	3.4×10^{-3}	1.5
4	15	11	0.50	2.8	3.7	2.2	28	26
5	15	33	4.8	2.8	10	11	>60	>64
8a	37	140	0.44	9.2	0.24	0.75	150	45
9	0.34	1.6	0.58	1.3×10^{-2}	0.40	1.2	NT	NT
9H	24	78	10	9.8	3.0	12	27	80
11	30	31	29	16	17	23	23	42
12	13	7.1	3.0	1.7	39	6.4	2.4	28
20	21	55	1.4	3.4	23	9.5	90	73
4H	31	89	7.5	15	580	88	39	70
13	17	6.5	2.1	2.4	43	23	5.6	13
21	105	55	1.6	19.3	5.6	30	130	100
18	5.9	NT	7.8	3.1	14	14	14	76
O_1I_N (**2**)	2.9×10^{-3}	0.62	0.22	1.1×10^{-3}	1.1×10^{-2}	2.4×10^{-2}	NT	NT
O_1G_N (**3**)	0.10	1.0	>2.6	3.1×10^{-2}	0.30	>2.2	NT	NT
cstat 1 (**19**)[6]	2.0×10^{-9}	NT	7.6×10^{-7}	1.7×10^{-8}	1.4×10^{-7}	4.0×10^{-8}	2.6×10^{-6}	4.1×10^{-4}

The activities of several intermediates offer further insight into this question. We note that 3-acetylated compounds generally showed cytoxicities significantly lower than their counterparts with a free 3-OH (cf. **5/9**, **11/12**, **4H/13**). Acetate **5**, which retains the 16β-OH moiety, has available the same C22 acyclic oxocarbenium ion pathways and indirect access to an E-OCI as does **9** (Fig. 6). Acetate **4** (16-keto) has even poorer access to an E-OCI form (multiple steps via a ketone hydrate, Fig. 7), yet was slightly *more* active than **5**. Indeed, **4** has the option of an acyclic C16 oxocarbenium ion in addition to that at C22. The saturated acetate **4H** (16-keto) was comparable to **9H** in potency, and **13** (deacetyl-**4H**) was more potent. Precursor **11** (16α-OH) may form an *acyclic* oxocarbenium ion, but access to an E-OCI by similar pathways seems unlikely, as this would entail formation of a highly strained *trans*-fused [3.3.0] bicyclic system.[16] Acetate **11** was only slightly less active than **9H**, and **12** (deacetyl-**11** = 16*epi*-**9H**) showed superior activity, suggesting that unavailability of (diversionary?) E-OCI formation pathways may enhance cytotoxicity. Notably, the unoxidized 22-OPMB ether precursors to **9**, whether 16α-OH or 16β-OH (not shown),[4] cannot form any oxocarbenium ion and were inactive even at 100 μM doses. These results suggest that an acyclic oxocarbenium ion is probable, and that a suitable 16-protected, 22-ketone form should also be active. Deketalization of **12** and **13** (cat. Pd^{2+}, see Fig. 3)[12] gave ketone **20** and diketone **21**, respectively (not shown). These free 22-ketones[16] did not display enhanced cytotoxicity, which validates the use of a ketal form for the analogues.

Figure 7



The activities displayed by the cephalostatin analogues **2** and **3** versus that of aglycones **8a**, **9** and **9H** of OSW-1 are also consistent with the proposed role of the oxocarbenium ions. Aglycone ketal **9** retains submicromolar activity, confirming that the steroid nucleus contributes to the bioactivity of OSW-1 (**1**). The

diminished potencies displayed by **5** (the 3β-acetate of **9**), by the related 3β-TBS ether[4] (not shown, completely inactive against all cell lines tested), and by **9H** (loss of the Δ^5 function) may indicate a biochemical role for the homoallylic alcohol in **1** as well as a topographical purpose (A/B ring-flattening, Fig. 8). Dihydro-ornithostatin O_1I_N (**2**), the union of North I and saturated aglycone ketal **9H**, displayed nanomolar activity and was up to 8,000 times more cytotoxic than the free dihydro-aglycone. The North I unit, as the free (3β-OH) pentaol, was inactive at 100 μM doses against the same cell lines in tests at the NCI.[14] In this connection, it is interesting to note that the North G unit was inactive as the 3-acetate or 3-ketone[6] but displayed moderate activity as the 12-acetate-aminomethoxime **18**. Although the analogues do not attain the level of cytoxicity displayed by cephalostatin 1 (**19**, Fig. 8), the dramatic enhancement in potency by formation of the pyrazine **2** supports the argument that the mode of bioactivity of these two families are indeed closely related.

In addition to the flattened A/B rings, we note that **2** and **3** retain homoallylic alcohols in the C/D rings of their North hemispheres (Fig. 2). While dihydro-ornithozine O_1G_N (**3**), the union of North G and the aglycone **9H**, also showed significant improvement (up to 300-fold) relative to the free aglycone, the superior cytotoxicity displayed by **2** vs. **3** confirms the"matching requirement" for high potency seen for the subunit pairs of all compounds thus far reported.[6] This may reflect a desirable polarity difference as originally proposed by Fusetani[2] (i.e., North I is sufficiently more polar than **9H**, while North G is too similar) or a more subtle requirement, such as a need for a 17α-hydroxyl in the same subunit as the homoallylic alcohol array.

A long-standing question has been, "what role does the central pyrazine ring play in biological activity?" The free (3-OH) North I and South 7[17] subunits were found to be non-cytotoxic, probably due to the absence of the central pyrazine ring. When fused via such a pyrazine ring, the trisdecacyclic steroid "dimers" average about 30 Å in overall length (~23 Å between spiroketal centers). This dimension, about half the depth of a cell

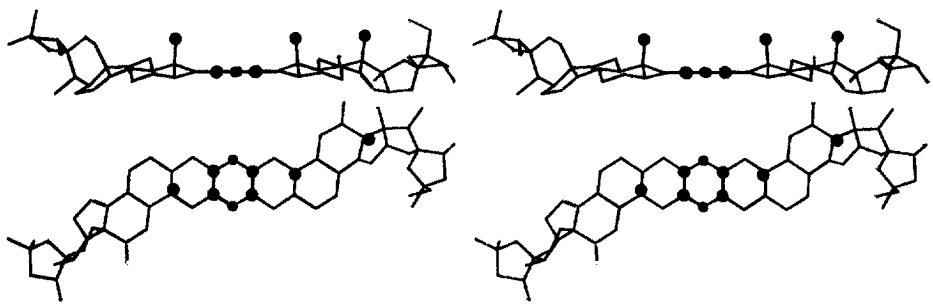
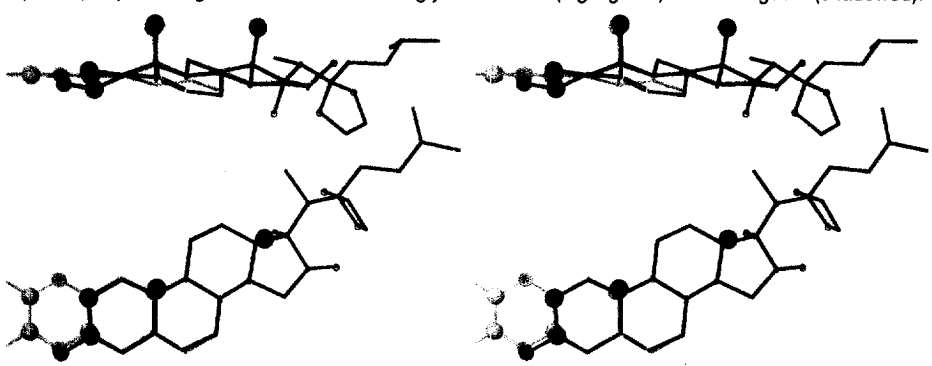


Figure 8 (Above) Top and edge 3D views of cephalostatin 1 (**19**).
(Below) Top and edge views of the OSW-1 aglycone ketal **9** (highlighted) and analogue **2** (shadowed).



membrane, nicely spans the membrane's "high" and "low" electron density regions.[18] Such a topological feature may help explain the observed higher potency for compounds with a matched "nonpolar" and "polar" pair of subunits. As seen in cephalostatin 1 (**19**, Fig. 8), both sets of the nonpolar A/B rings in the steroid units lie mainly in the pyrazine plane. This unique structural motif might further facilitate entry into cell membranes. The OSW-1 aglycone ketal **9** also features a "flattened" A/B ring set which, with its polar 3βOH group, is quite similar to that of cholesterol, an integral component of cell membranes. Ready passage into the membrane thus also seems likely for OSW-1 (**1**). The role of its disaccharide moiety remains to be elucidated.

In conclusion, OSW-1 and the cephalostatin family appear to share similar modes of action, and the SAR data of our synthetic compounds indicates that an acyclic oxocarbenium ion, which is more readily generated from a ketal than the parent ketone, is the likely intermediate responsible for cytotoxicity. Further definition of the minimum pharmacophore and exploration of the site of activity are under active investigation.

Acknowledgments: We thank the NIH (CA 60548) and the Purdue Cancer Center (CG, TGL fellowships) for financial support, and Professor Sashida for kindly providing biological activity data and a sample of OSW-1.

References and Notes

Cephalostatin Support Studies 15. For Paper 14 in this series, see ref 17.
1. Pettit, G. R.; Tan, R.; Xu, J.-P.; Ichihara, Y.; Williams, M. D.; Boyd, M. R. *J. Nat. Prod.* **1998**, *61*, 953.
2. Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1997**, *62*, 4484 and references cited therein.
3. Mimaki, Y.; Kuroda, M.; Kameyama, A.; Sashida, Y.; Hirano, T.; Oka, K.; Maekawa, R.; Wada, T.; Sugita, K.; Beutler, J. A. *Bioorg. & Med. Chem. Lett.* **1997**, *7*, 633 and references therein.
4. Guo, C.; Fuchs, P. L. *Tetrahedron Lett.* **1998**, *39*, 1099.
5. LaCour, T. G.; Guo, C.; Boyd, M. R.; Fuchs, P. L. *Bioorg. Med. Chem. Lett.* submitted.
6. LaCour, T. G.; Guo, C.; Bhandaru, S.; Boyd, M. R.; Fuchs, P. L. *J. Am. Chem. Soc.* **1998**, *120*, 692.
7. Kim, S.; Sutton, S.; Guo, C.; LaCour, T. G.; Fuchs, P. L. *J. Am. Chem. Soc.*, in press, and refs 6 and 13.
8. The naming system reflects the origins of the steroidal units: 'Ornitho' from Ornithogalum saundersiae, 'statin' from cephalostatin and 'ritter' or 'zine' from ritterazine, in keeping with the system adopted for the earlier hybrid ritterostatins (ref 6).
9. The NMR of **8a** showed a single diastereomer and was consistent with that of related 22*S* compounds. The 22*S* configuration was also calculated to be 2.2 kcal/mol more stable than the 22*R* epimer (CAChe v.3.5).
10. All new compounds gave satisfactory ^{1}H and ^{13}C NMR spectra, MS and HRMS.
11. Li, C.; Shih, T. L.; Jeong, J. U.; Arasappan, A.; Fuchs, P. L. *Tetrahedron Lett.* **1994**, *35*, 2645.
12. Reuss, R. H.; Hassner, A. *J. Org. Chem.* **1974**, *39*, 1785.
13. Guo, C.; Bhandaru, S.; Fuchs, P. L.; Boyd, M. R. *J. Am. Chem. Soc.* **1996**, *118*, 10672.
14. Mosmann, T. *J. Immuno. Methods* **1983**, *65*, 55-63, and Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.
15. TLC indicated some formation of **8b** when a chloroform solution of **8a** was allowed to stand for 20h.
16. Conceivably, protonation of the 16αOH in **11** or **12** and β face attack by ketal or ketone oxygen could lead to a hemiketal and an E-ring oxocarbenium ion. However, we have observed no evidence for such reaction in either **12** nor **20** upon standing in acidic chloroform.
17. (a) Jeong, J. U.; Fuchs, P. L. *Tetrahedron Lett.* **1995**, *36*, 2431; (b) Jeong, J. U.; Guo, C.; Fuchs, P. L. *J. Am. Chem. Soc.* in press.
18. Caspar, D. L. D. and Kirschner, D. A. *Nature:NB* **1971**, *231*, 46.