JOC Article

Synthesis of Bis-18,18′-desmethyl Ritterazine N

Douglass F. Taber* and Jean-Michel Joerger

Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716.

taberdf@udel.edu

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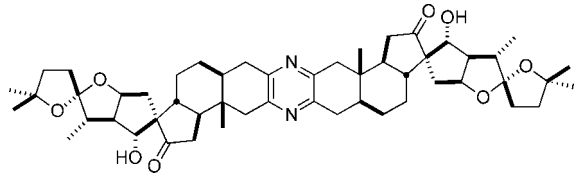

Bis-18,18′-desmethyl ritterazine N has been prepared in enantiomerically pure form. The synthetic alkaloid, lacking only two of the 52 carbon atoms of the natural product, shows selective activity in the NIH 60 cell panel.

Introduction

The ritterazines (represented by ritterazine N **1**), found in small quantities in the lipophilic extract of the tunicate *Ritterella tokioka*, induce apoptosis in apoptosis-resistant malignant cells.[1] With the closely related cephalostatins, which show the same activity, they form a unique class of trisdecacyclic molecules featuring a pyrazine as the core ring, steroid-related structures, and spiroketal edge-rings (E and F). Partial syntheses from steroid precursors of several of the 6-6-6-5 cephalostatins and derivatives have been accomplished.[2] There has been no report of efforts other than our own toward the 6-6-5-5 ritterazines. In preceding papers, we described[3] the preparation of the A−B−C carbocyclic core **2** of these ritterazines, and the preparation[4] of the 5/5-spiroketal **3** (rings E and F, rings E′ and F′). We describe here the assembly of bis-18,18′-desmethyl ritterazine N **4**, and an initial report of the activity of **4** in the NCI 60 cell panel (Figure 1).

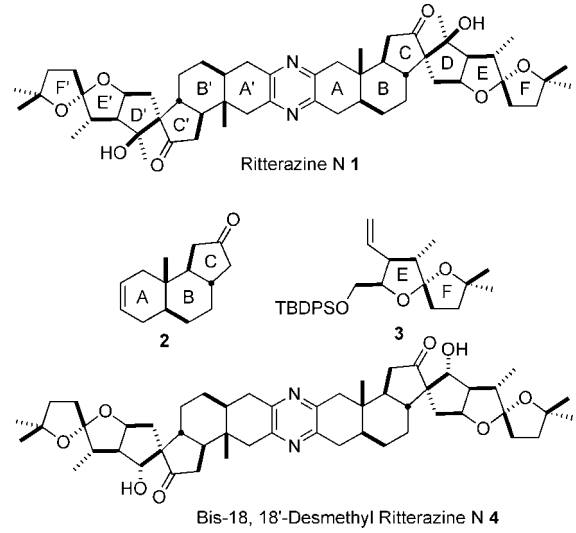

FIGURE 1. Structures of ritterazines and precursors.

Results and Discussion

Preparation of the Enantiomerically Pure Tricyclic Ketone. The ketone **2** we had previously prepared was racemic. To prepare the enantiomerically pure ketone **8**, we exposed racemic **2** to mandelic acid and *N*-bromosuccinimide in the presence of 2,6-lutidine (Scheme 1). As expected,[5] just two diastereomeric bromomandelates were formed, the product of Br^+ complexation to the more accessible face of the alkene followed by diaxial opening with mandelate anion.

The diastereomeric mandelates were separated by column chromatography. The structures were assigned by ^{1}H NMR analysis, following our earlier precedent.[5] This assignment was confirmed by X-ray analysis of the mesylate **11** (Scheme 2).

Saponification of the bromomandelate **6** led directly to the β ("up") epoxide **8**. We had previously shown[3] that the

(1) For the isolation and activity of the ritterazines and cephalostatins, see: (a) Fusetani, S.; Fukuzawa, S.; Matsunaga, S. *J. Org. Chem.* **1997**, *62*, 4484. (b) Pettit, G. R.; Tan, R.; Xu, J.; Ichihara, Y.; Williams, M. D.; Boyd, M. R. *J. Nat. Prod.* **1998**, *61*, 955. (c) Komiya, T.; Fusetani, N.; Matsunaga, S.; Kubo, A.; Kaye, F. J.; Kelley, M. J.; Tamura, K.; Yoshida, M.; Fukuoka, M.; Nakagawa, K. *Cancer Chemother. Pharmacol.* **2003**, *51*, 202. (d) Lopez-Anton, N.; Rudy, A.; Barth, N.; Schmitz, L. M.; Pettit, G. R.; Schulze-Osthoff, K.; Dirsch, V. M.; Vollmar, A. M. *J. Biol. Chem.* **2006**, *281*, 33078.

(2) For leading references, see: (a) Lee, J. S.; Fuchs, P. L. *J. Am. Chem. Soc.* **2005**, *127*, 13122. (b) Nawasreh, M.; Winterfeldt, E. *Curr. Org. Chem.* **2003**, *7*, 649.

(3) Taber, D. F.; Taluskie, K. V. *J. Org. Chem.* **2006**, *71*, 2797.

(4) (a) Taber, D. F.; Joerger, J.-M. *J. Org. Chem.* **2007**, *72*, 3454. (b) While our work was in press, Shair reported reaching the same conclusion about the relative configuration of the spiroketal: Phillips, S. T.; Shair, M. D. *J. Am. Chem. Soc.* **2007**, *129*, 6589.

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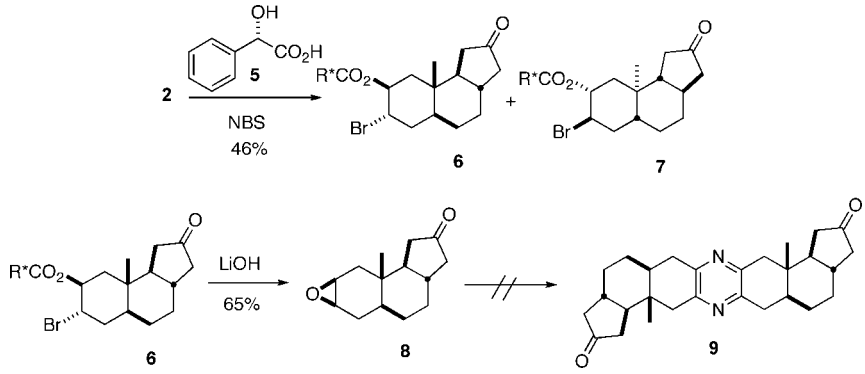
SCHEME 1



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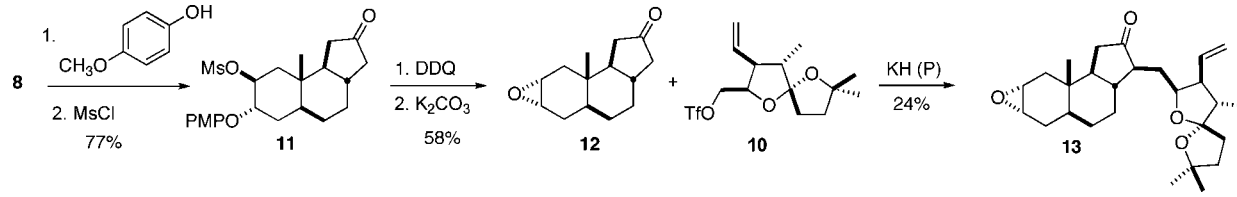

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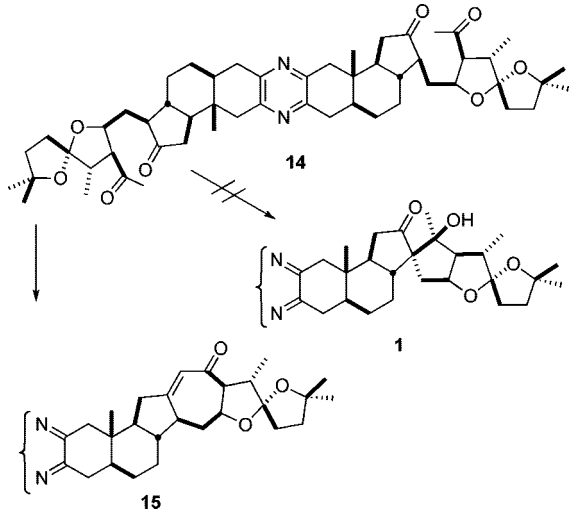
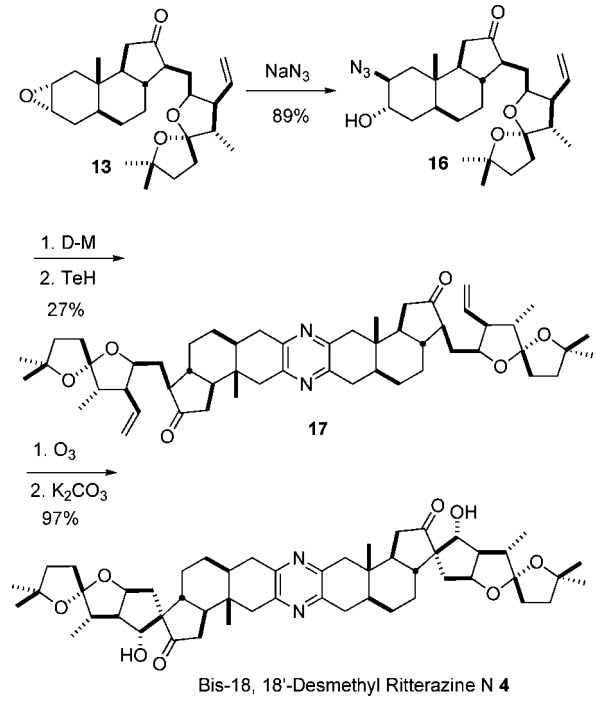


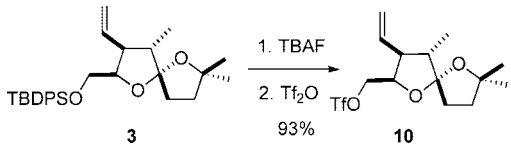
SCHEME 4



Bis-18, 18'-Desmethyl Ritterazine N **4**

analogous α epoxide, from direct epoxidation of **2**, could be dimerized to **9** by opening with azide, oxidation to the ketone, and reduction with Te/$NaBH_4$. To our surprise, only traces of **9** could be found when the same reductive protocol was applied to the azido ketone prepared from **8**. While it might be possible to find conditions for the dimerization from the β epoxide, we chose to convert the β epoxide to the easily dimerized α epoxide before proceeding with the synthesis.

Preparation of the Spiroketal Triflate. The spiroketal **3** that we had previously prepared[4a] was converted to the triflate **10** by desilylation followed by sulfonylation with triflic anhydride (eq 1). We also prepared the iodide corresponding to **10**, but this proved to be a less efficient alkylating agent than **10**.



(1)

Preparation and Alkylation of the Epoxy Ketone. The epoxide of **8** was inverted by opening[5] with 4-methoxyphenol, followed by mesylation, to give **11**. The mesylate **11** gave crystals that were suitable for X-ray analysis, confirming the previously assigned absolute configuration. Oxidative removal of the aryl ether followed by cyclization then delivered the α epoxide **12**.

The alkylation of **12** was challenging. We anticipated that we could arrive at **13** by kinetic deprotonation of the more accessible methylene of **12**. In the event, the lithium enolate, prepared by exposing **12** to LDA, was not sufficiently reactive toward **10**, even at room temperature and above. We eventually found that exposure of **12** to KH, conveniently delivered as KH

TABLE 1. Activity of 1 in the National Cancer Institute 60 Cell Panel

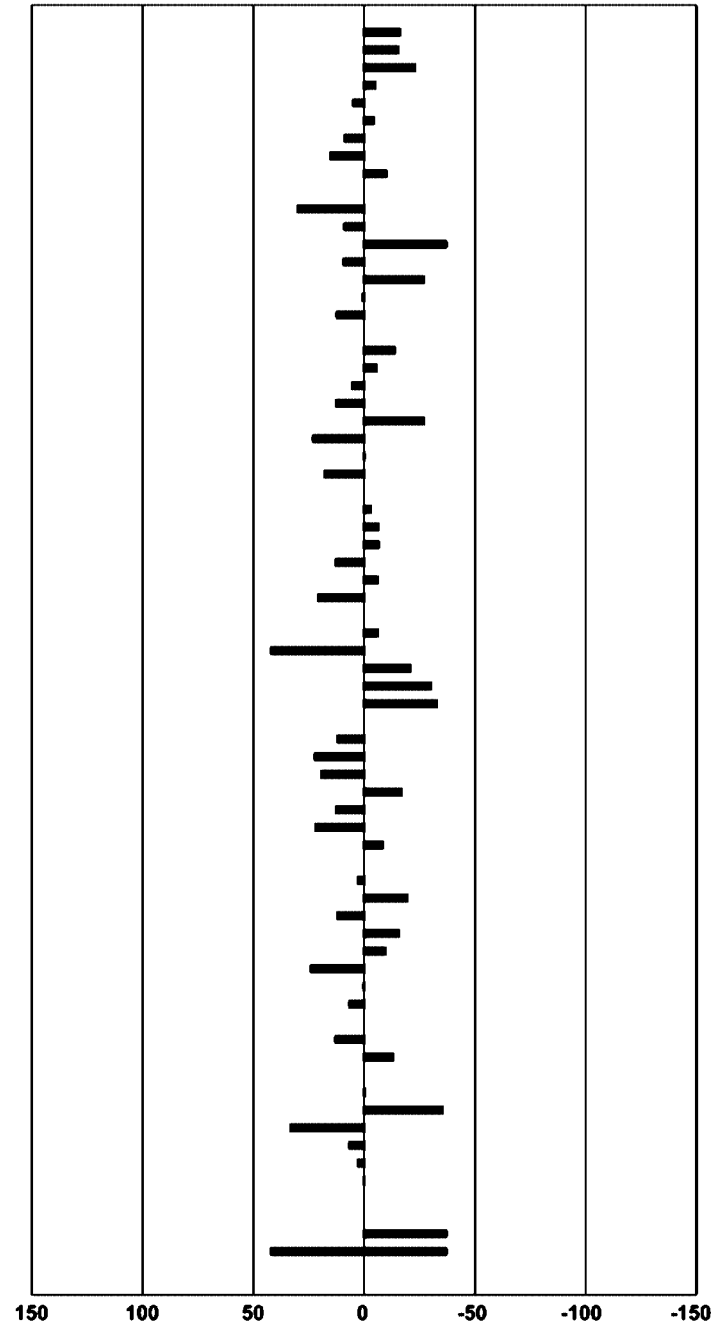
Developmental Therapeutics Program

One Dose Mean Graph

NSC: 746394 / 1	Conc: 1.00E-5 Molar	Test Date: Oct 22, 2007
Experiment ID: 0710OS38		Report Date: Nov 13, 2007

Panel/Cell Line	Growth Percent
Non-Small Cell Lung Cancer	
A549/ATCC	82.64
EKVX	83.34
HOP-62	75.69
HOP-92	93.64
NCI-H226	104.01
NCI-H23	94.47
NCI-H322M	107.88
NCI-H460	114.23
NCI-H522	88.72
Colon Cancer	
COLO 205	128.95
HCC-2998	108.00
HCT-116	61.59
HCT-15	108.16
HT29	71.83
KM12	99.80
SW-620	111.29
Breast Cancer	
BT-549	84.87
HS 578T	93.12
MCF7	104.43
MDA-MB-231/ATCC	111.50
MDA-MB-435	71.68
MDA-MB-468	121.97
NCI/ADR-RES	98.82
T-47D	116.79
Ovarian Cancer	
IGROV1	95.65
OVCAR-3	92.34
OVCAR-4	92.09
OVCAR-5	111.85
OVCAR-8	92.57
SK-OV-3	119.74
Leukemia	
CCRF-CEM	92.55
HL-60(TB)	141.05
K-562	77.78
MOLT-4	68.62
RPMI-8226	65.84
Renal Cancer	
786-0	111.10
A498	121.29
ACHN	118.26
CAKI-1	81.74
SN12C	111.65
TK-10	120.98
UO-31	90.29
Melanoma	
LOX IMVI	101.63
M14	79.23
MALME-3M	111.09
SK-MEL-2	83.20
SK-MEL-28	89.07
SK-MEL-5	123.12
UACC-257	99.29
UACC-62	105.59
Prostate Cancer	
DU-145	111.96
PC-3	85.73
CNS Cancer	
SF-268	98.50
SF-295	63.30
SF-539	132.24
SNB-19	105.61
SNB-75	101.84
U251	99.17
Mean	98.85
Delta	37.26
Range	79.46

Mean Growth Percent - Growth Percent



in paraffin,[6] generated an enolate that reacted nearly quantitatively with the triflate **10**.

Successfully reacting **12** with **10** was not the end of the difficulties. The product was a mixture of both regioisomeric C-alkylation products and also enol ethers from O-alkylation. It was necessary to develop conditions for acidic hydrolysis of the O-alkylated byproducts without upsetting the acid-sensitive spiroketal. We found success by stirring the crude alkylated mixture with $CDCl_3$ (nonstabilized chloroform) containing a little bit of aqueous HCl. The regenerated **12** could then be separated from the alkylated product **13** and from the alkylated regioisomer by column chromatography.

The Aldol Condensation Fails. We had originally envisoned (Scheme 3) that the diketone **14** could cyclize to the aldol

product **1**. In the event, through a range of bases and solvents, we were not able to detect **1** in the crude reaction mixtures. Rather, the product appeared to be predominantly the cycloheptenone **15**. We are investigating alternative strategies for the preparation of **1**.

Preparation of Bis-18,18′-desmethyl Ritterazine N 4. In the course of our investigations, we prepared (Scheme 4) the dimerized pyrazine **17**. Diaxial opening of **13** with sodium azide delivered the alcohol **16**. The ketone from the oxidation of **16** was not stable, so we submitted it directly to dimerization conditions,[7] to give **17**.

We were pleased to observe that ozonolysis followed by brief exposure to base led to clean aldol condensation, to deliver bis-18,18′-desmethyl ritterazine N **4** as a single diastereomer.[8] As an interim step in our investigations, we submitted the synthetic **4** for analysis in the NIH 60-cell screen. In fact, **4** did show (Table 1) modest differential activity across the several cell lines. It would be interesting to compare these data to those for ritterazine N **1** itself. Unfortunately, that substance is not currently available.

Conclusion

We are pleased that we were able to prepare practical quantities of both the enantiomerically pure ketones **8** and **12**, and the triflate **10**, and that we were able to alkylate the ketone **12** with the triflate **10**. The capability to dispense scrupulously dry KH in paraffin in micromole quantities was critical for the success of this alkylation. For the first time, this makes derivatives such as **4**, having the full ring framework of the 6-6-5-5 ritterazines, available for further evaluation.

Experimental Section

Alkylated Ketone 13. To ketone **12** (234 mg, 1.06 mmol), azeotropically dried with toluene, was added KH (128 mg of 50% w/w mixture of KH/paraffin, 1.59 mmol of KH)[7] and THF (8 mL). After 1 h at rt, the triflate **10** (190 mg, 0.530 mmol) in THF (8 mL) was added. After 30 min at rt, the reaction mixture was partitioned between EtOAc and saturated aqueous NH_4Cl. The organic extract was dried (Na_2SO_4) and concentrated. To the residue were added $CDCl_3$ (8 mL) and 3 drops of 0.1 M aqueous HCl. The mixture was stirred for 1.5 h, then partitioned between EtOAc, and, sequentially, saturated aqueous $NaHCO_3$ and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to give the alkylated product **13** as a colorless oil (20.0 mg, 9% yield based on starting triflate, 24% yield based on recovered ketone), recovered ketone **12** (82%), and the spiro alcohol (43%). **13**: TLC R_f (MTBE/PE = 20:80) 0.36; ^{1}H NMR δ 0.80 (s, 3H), 0.86 (d, J = 6.7 Hz, 3H), 1.10−1.36 (m, 8H) includes {1.14 (s, 3H), 1.31 (s, 3H)}, 1.42−2.12 (m, 18H), 2.23 (dd, J = 16.8, 4.9 Hz, 1H), 2.73 (m, 1H), 3.10 (dd, J = 5.8, 4.1 Hz, 1H), 3.17−3.21 (m, 1H), 4.37 (m, 1H), 5.03−5.13 (m, 2H), 5.65 (dt, J = 16.9, 9.8 Hz, 1H); ^{13}C NMR δ ϵ[9] u 219.7, 117.6, 115.2, 81.6, 44.7, 39.0, 37.8, 34.6, 33.2, 32.1, 32.0, 29.0, 28.8, d 136.5, 75.0, 53.3, 52.4, 52.0, 50.5, 47.5, 43.4, 36.4, 36.0, 30.1, 28.3, 12.8, 11.2; IR (cm^{-1}) 2967, 2921, 1739, 999; MS m/z (%) 451 (M + Na, 100), 243 (6); HRMS calcd for $C_{27}H_{40}O_4Na$ (M + Na) 451.2824, obsd 451.2815; $[\alpha]_D$ +22 (c 0.36, CH_2Cl_2).

Azido Alcohol 16. In a sealable tube were combined epoxide **13** (20.0 mg, 46.6 μmol), sodium azide (33 mg, 500 μmol), and a methanol/water solution (8:1, 1 mL). The tube was sealed and heated. After 5 h at 102 °C, the reaction mixture was cooled, then partitioned between EtOAc, and sequentially, water and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to give the azido alcohol **16** as a colorless oil (19.6 mg, 89%). TLC R_f (MTBE/PE = 40:60) 0.46; ^{1}H NMR δ 0.87 (d, J = 6.7 Hz, 3H), 1.03 (s, 3H), 1.15 (s, 3H), 1.19−1.44 (m, 7H) includes 1.33 (s, 3H), 1.47−2.14 (m, 17H), 2.20−2.28 (m, 1H), 2.72 (m, 1H), 3.77 (m, 1H), 3.92 (m, 1H), 4.38 (m, 1H), 5.05−5.13 (m, 2H), 5.65 (m, 1H); ^{13}C NMR δ u 219.5, 117.7, 115.3, 81.7, 44.7, 37.8, 36.8, 36.7, 33.2, 32.3, 32.1, 31.6, 28.5, d 136.4, 75.1, 68.4, 61.3, 54.3, 52.0, 47.6, 43.5, 38.7, 35.4, 30.1, 28.3, 12.3, 11.2; IR (cm^{-1}) 3439 (br), 2923, 2102, 1734, 1000; MS m/z (%) 494 (M + Na, 100); HRMS calcd for $C_{27}H_{41}N_3O_4Na$ (M + Na) 494.2995, obsd 494.2985; $[\alpha]_D$ −35 (c 0.70, CH_2Cl_2).

Pyrazine 17. To a solution of azido alcohol **16** (14.0 mg, 39.7 μmol) in CH_2Cl_2 (1 mL) was added Dess−Martin periodinane (85 mg, 200 μmol) at rt. After 2 h at rt, a 2 mL mixture of saturated aqueous $NaHCO_3$, saturated aqueous $Na_2S_2O_3$, and water (1:1:1) was added to the reaction mixture. The resulting mixture was stirred for an additional 5 min, then was partitioned between MTBE and brine. The organic extract was dried (Na_2SO_4) and concentrated to crude azido ketone. A solution of NaTeH (approximatively 0.25 M) was prepared by heating powdered tellurium (510 mg, 4 mmol) and $NaBH_4$ (378 mg, 10 mmol) to 75 °C in ethanol (16 mL) for 1 h. A 0.033 M solution of NaTeH was prepared by adding 4 mL of the 0.25 M solution to 26.3 mL of ethanol thoroughly degassed with N_2. To the crude azido ketone was added 2 mL (66 μmol) of the freshly prepared 0.033 M solution of NaTeH, and the resulting suspension was stirred for 1 h at rt under N_2, then overnight at rt under O_2. The mixture was partitioned between CH_2Cl_2 and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to give the pyrazine **17** as a white solid (3.4 mg, 27%). Mp 244−246 °C dec; TLC R_f (MTBE/PE = 50:50) 0.46; ^{1}H NMR δ 0.83 (s, 6H), 0.87 (d, J = 6.7 Hz, 6H), 1.14 (s, 6H), 1.23−1.51 (m, 10H) includes 1.33 (s, 6H), 1.64−2.25 (m, 28H), 2.31 (dd, J = 16.4, J = 6.5 Hz, 2H), 2.58 (dd, J = 17.7, 12.3 Hz, 2H), 2.66−2.93 (m, 8H), 4.42 (m, 2H), 5.06−5.15 (m, 4H), 5.70 (m, 2H); ^{13}C NMR δ u 219.4, 148.5, 148.4, 117.8, 115.3, 81.6, 46.1, 44.6, 37.8, 36.4, 35.3, 33.1, 32.0, 31.7, 29.1, d 136.2, 74.9, 53.2, 52.0, 47.7, 43.5, 41.9, 36.0, 30.1, 28.3, 11.7, 11.2; IR (cm^{-1}) 2967, 2924, 1739, 1002; MS m/z (%) 872 (M + Na), 447 (29), 399 (63); HRMS calcd for $C_{54}H_{76}N_2O_6Na$ (M + Na) 871.5601, obsd 871.5596; $[\alpha]_D$ +16 (c 0.17, CH_2Cl_2); UV (MTBE) λ_{max} 289 (ϵ 11600).

Bis-18,18′-desmethylritterazine N 4. To bisalkene **14** (3.4 mg, 4.0 μmol) was added 0.1 mg of Sudan-III and 2 mL of CH_2Cl_2, and ozone was passed through the solution at −78 °C until the color turned from red to yellow. Excess ozone was removed by bubbling nitrogen through the solution. PPh_3 (10 mg, 40 μmol) was added, the solution was warmed to rt, and the solvent was evaporated. To the residue was added 2 mL of the upper phase from a mixture of {THF (3 volumes) + ethanol (3 volumes) + 10 wt % aqueous NaOH (1 volume)}. The mixture was stirred at rt for 1.5 h, then partitioned between EtOAc, and sequentially, half-saturated aqueous NH_4Cl and brine. The organic extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed to give the desmethylritterazine N **4** as a colorless oil (3.3 mg, 97%). TLC R_f (EtOAc) 0.45; ^{1}H NMR δ 0.99 (s, 6H), 1.10 (d, J = 6.7 Hz, 6H), 1.18 (s, 6H), 1.22−1.38 (m, 8H) includes 1.34 (s, 6H), 1.38−1.52 (m, 2H), 1.52−1.63 (m, 2H), 1.66−2.17 (m, 26H), 2.42−2.70 (m, 6H), 2.86 (dd, J = 18.0, 5.5 Hz, 2H), 2.96 (d, J =

(5) Taber, D. F.; Liang, J. *J. Org. Chem.* **2007**, *72*, 4313.

(6) For the advantages of KH in paraffin, see: Taber, D. F.; Nelson, C. G. *J. Org. Chem.* **2006**, *71*, 8973.

(7) (a) Suzuki, H.; Kawaguchi, T.; Takaoka, K. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 665. (b) Jeong, J. U.; Sutton, S. C.; Kim, S.; Fuchs, P. L. *J. Am. Chem. Soc.* **1995**, *117*, 10157.

(8) We have not yet had sufficient material for X-ray analysis of the synthetic **4**. The structure is assigned on the basis of aldol addition to the more open face of the cyclopentanone, to give the diastereomer of the secondary alcohol that can hydrogen bond to the ketone.

(9) ^{13}C multiplicities were determined with the aid of a JVERT pulse sequence, differentiating the signals for methyl and methine carbons as "d" from methylene and quaternary carbons as "u".

16.1 Hz, 2H), 3.06 (d, $J = 16.2$, 2H), 4.19 (m, 2H), 4.72 (m, 2H); ^{13}C NMR δ u 220.6, 148.3, 148.3, 120.0, 82.0, 68.2, 45.7, 45.7, 37.5, 37.3, 35.4, 34.9, 33.3, 32.8, 28.7, d 84.3, 77.9, 56.4, 55.5, 47.2, 42.9, 33.6, 30.0, 28.5, 13.6, 13.4; IR (cm^{-1}) 3411 (br), 2966, 2925, 1731, 1400, 999; MS *m*/*z* (%) 876 (M + Na, 30), 579 (100); HRMS calcd for $C_{52}H_{72}N_2O_8Na$ (M + Na) 875.5186, obsd 875.5186; $[\alpha]_D$ +52 (*c* 0.16, CH_2Cl_2); UV (MTBE) λ_{max} 289.5 (ϵ 12800).

Acknowledgment. We thank John Dykins for recording mass spectra (supported by NSF 0541775), Steve Bai for NMR assistance, Glenn Yap for the X-ray structures, and the NIH (GM 60287) for financial support of this work. We thank John A. Beutler of NCI for the 60 cell results.

Supporting Information Available: General experimental procedures, experimental procedures,^{1}H and ^{13}C spectra, and X-ray data for**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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